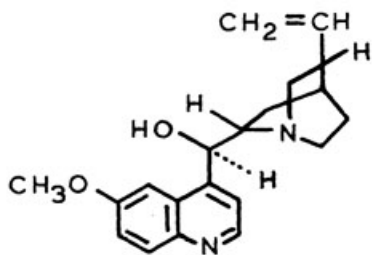
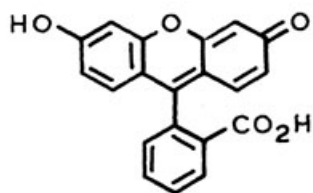


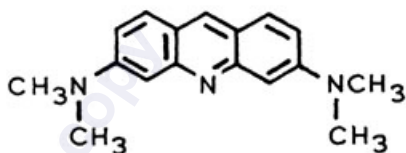
**POPOP**



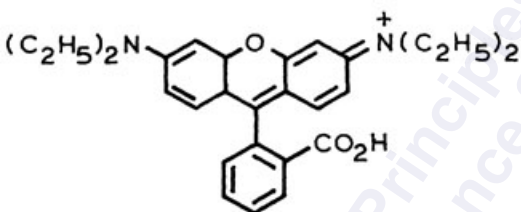
**Quinine**



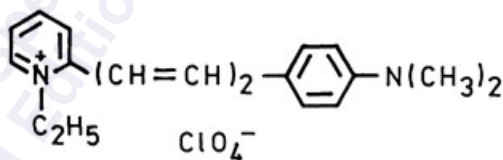
**Fluorescein**



**Acridine Orange**



**Rhodamine B**



**Pyridine 1**

POPOP

Quinine  
Sulphate

Fluorescein

Acridine  
Orange

Rhodamine B

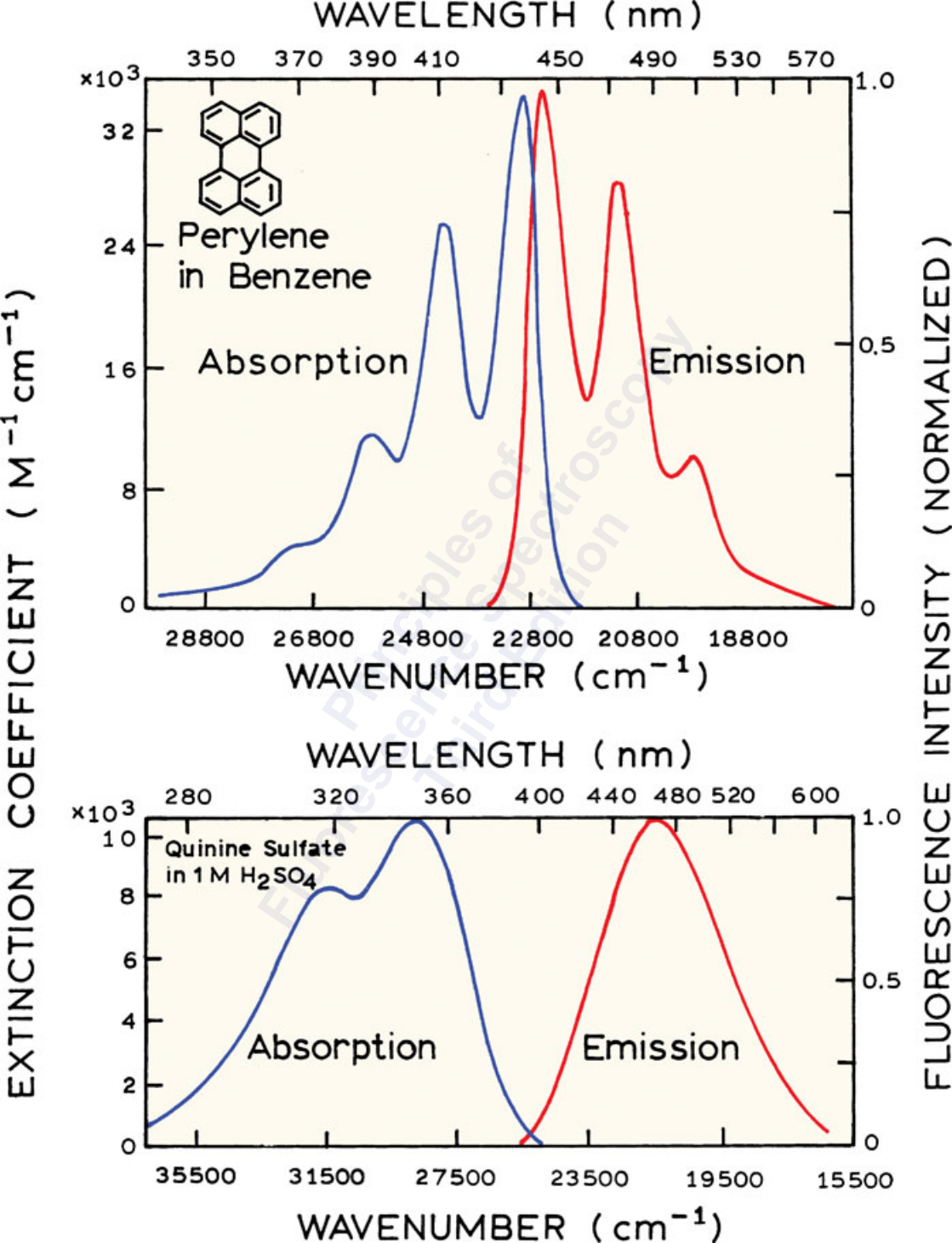
Pyridine 1



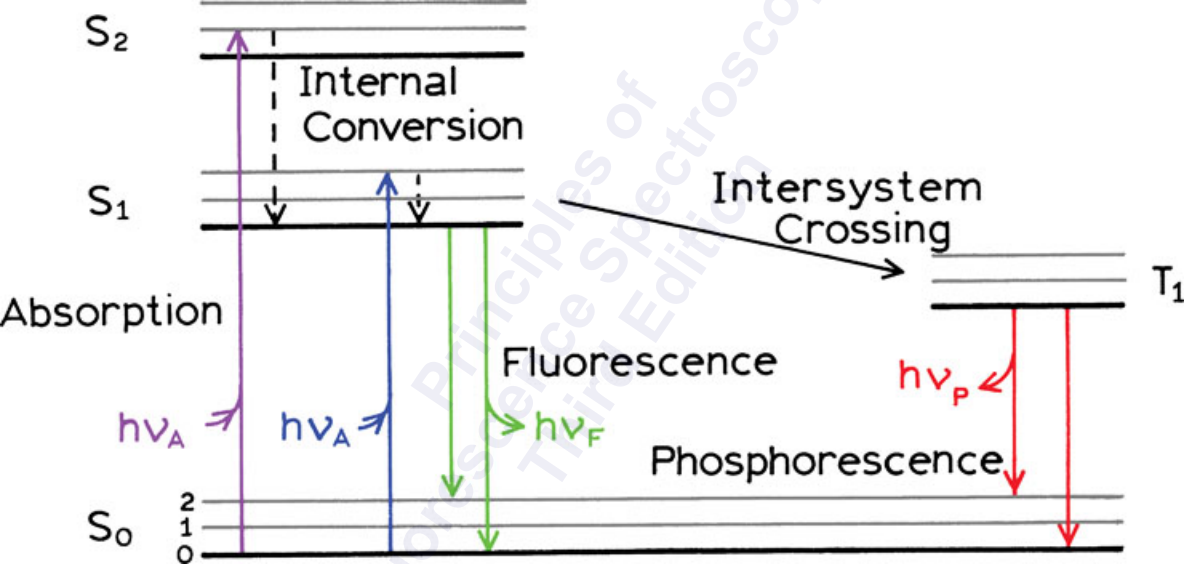
**FLUOROPHORES - UV to Red**

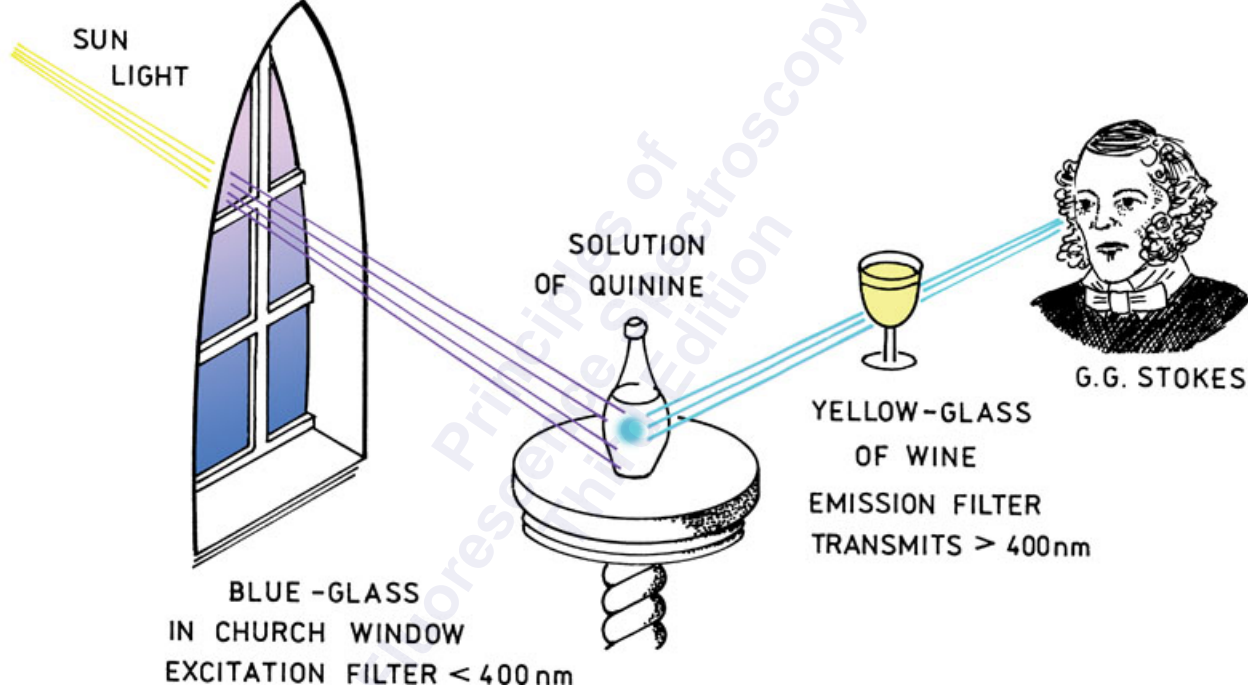




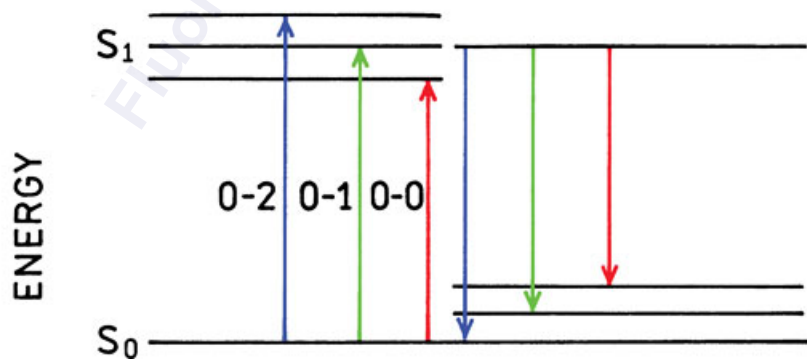
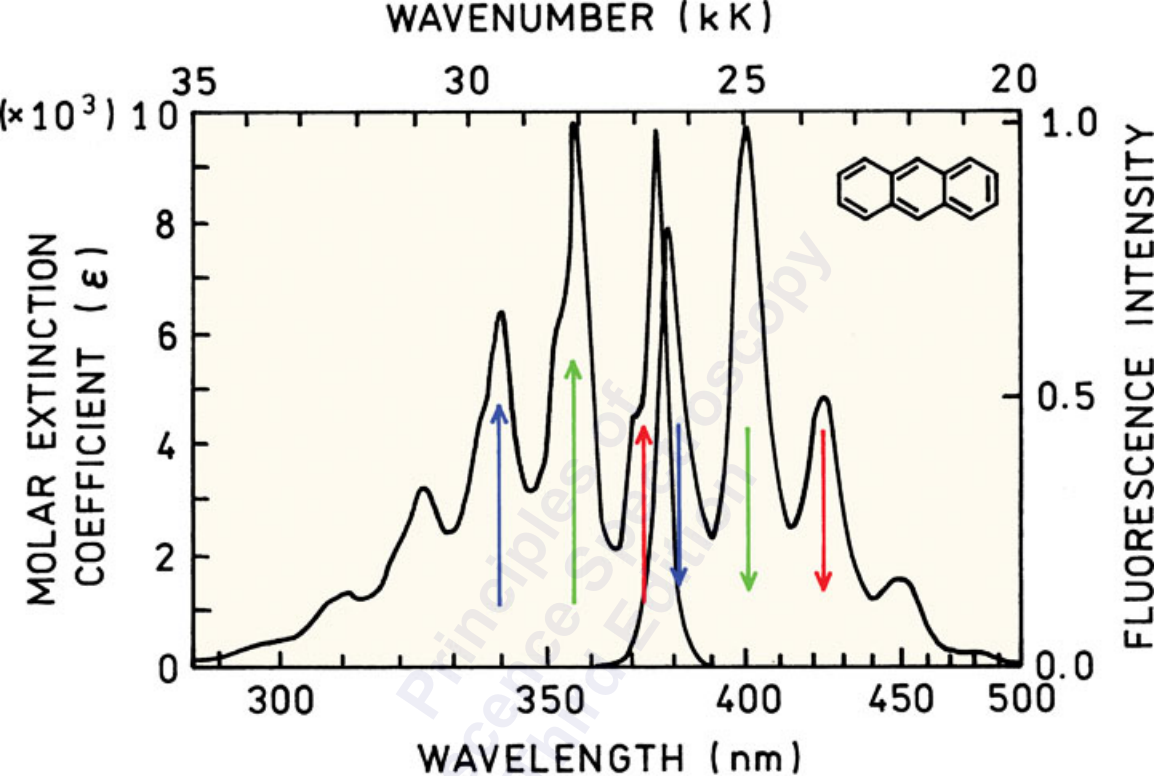




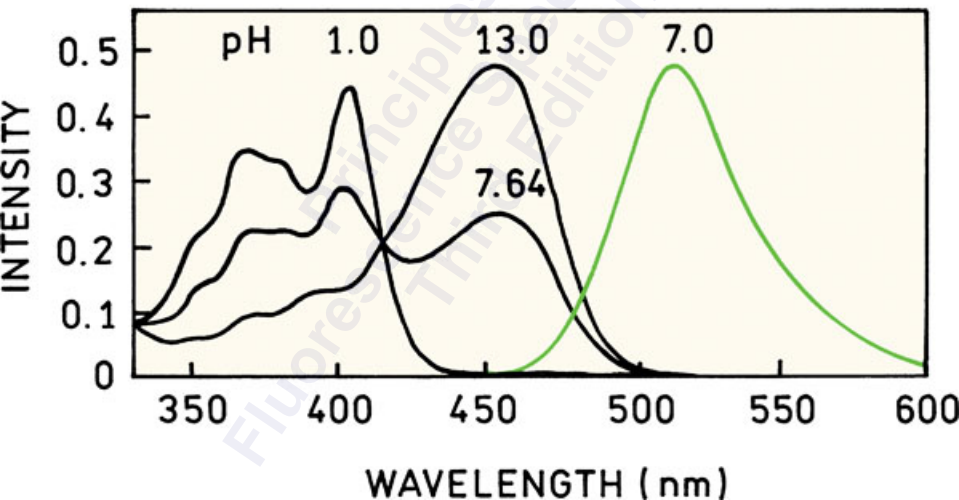
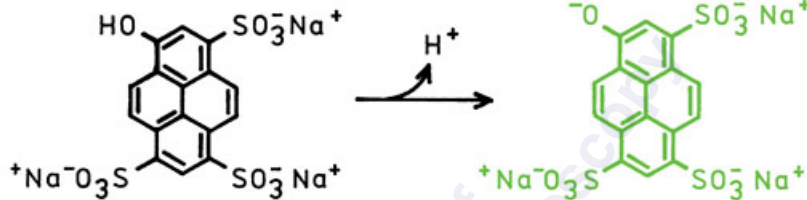


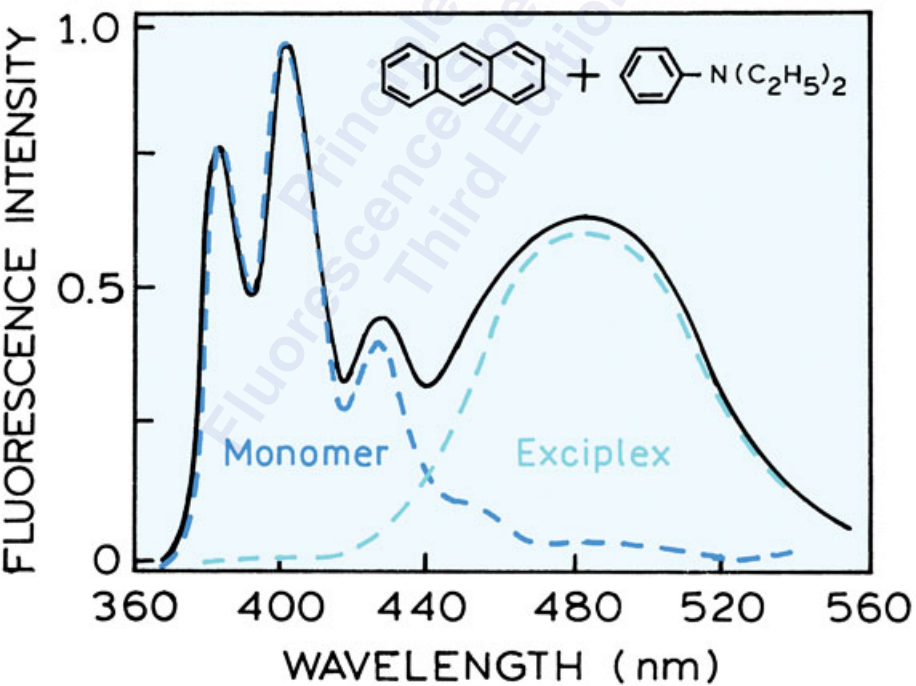
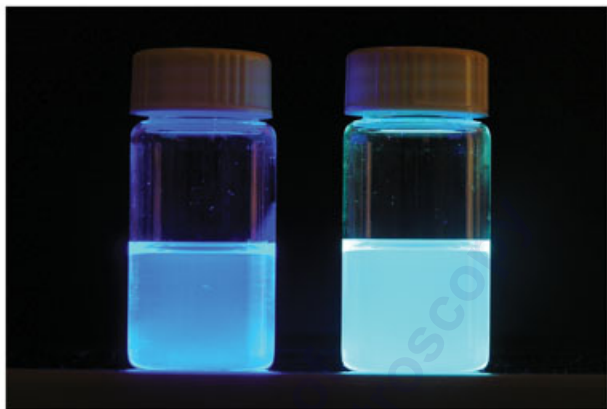


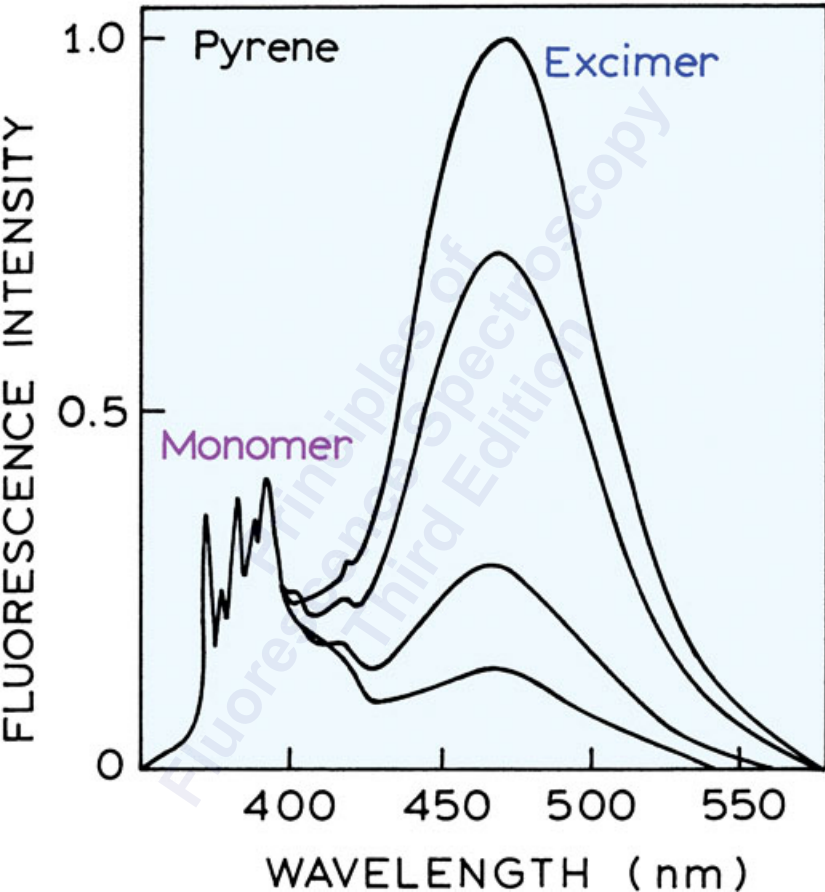


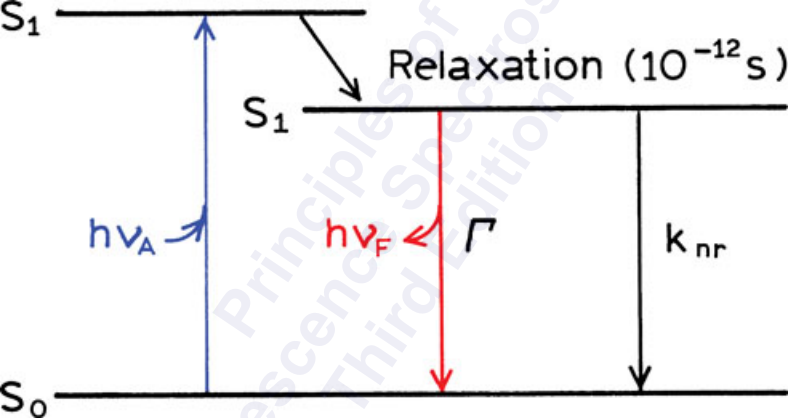




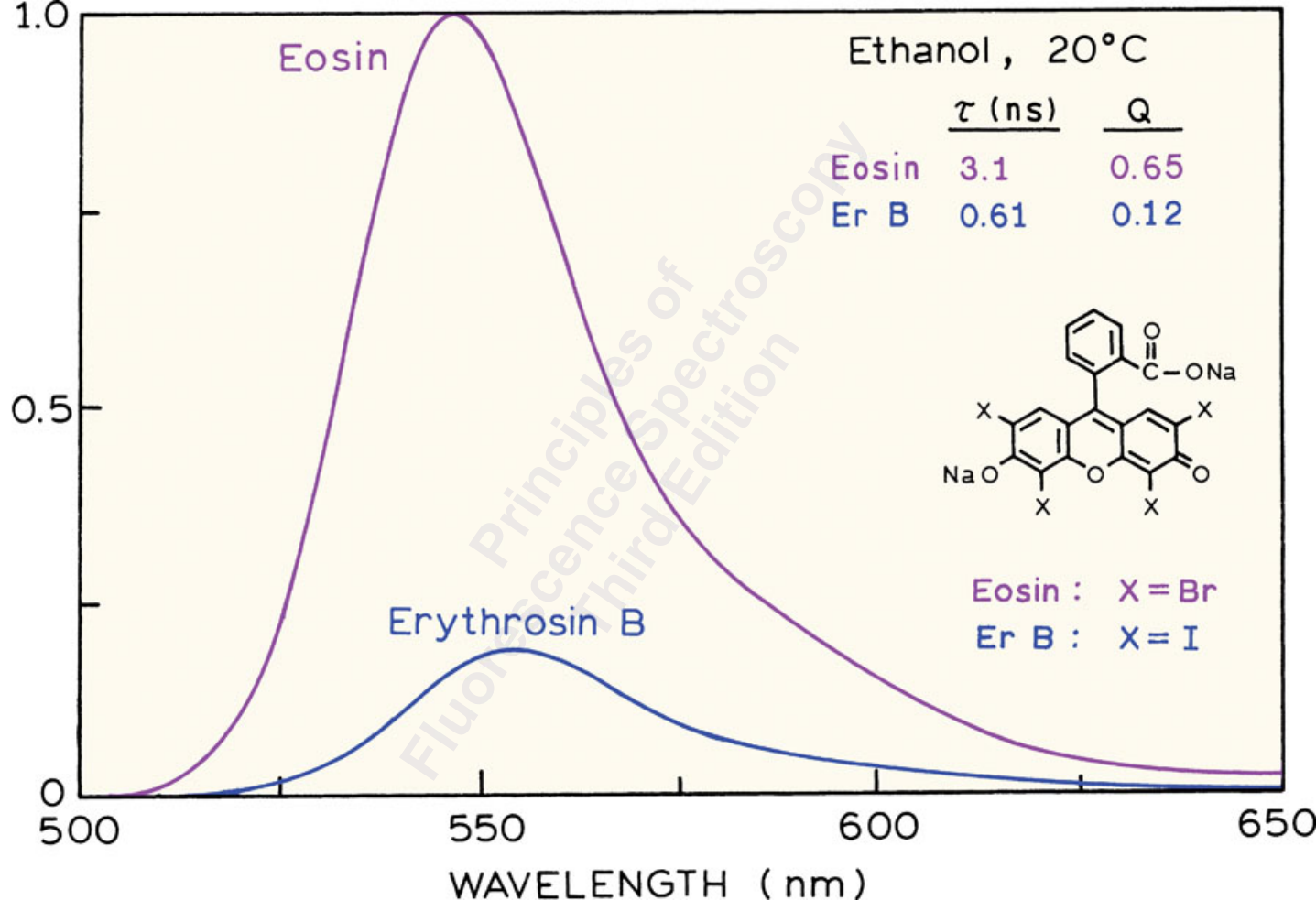


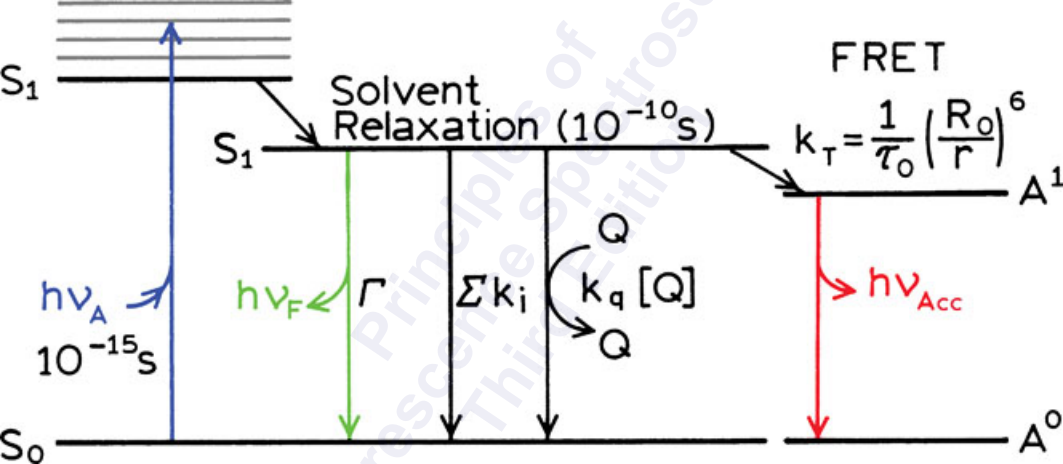






FLUORESCENCE INTENSITY



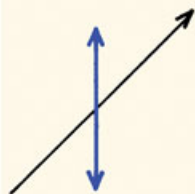




Photoselected  
fluorophores

Randomized  
fluorophores

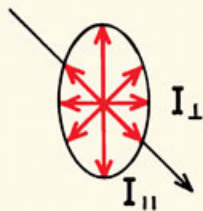
Rotational  
diffusion



Polarized  
excitation



Polarized  
emission



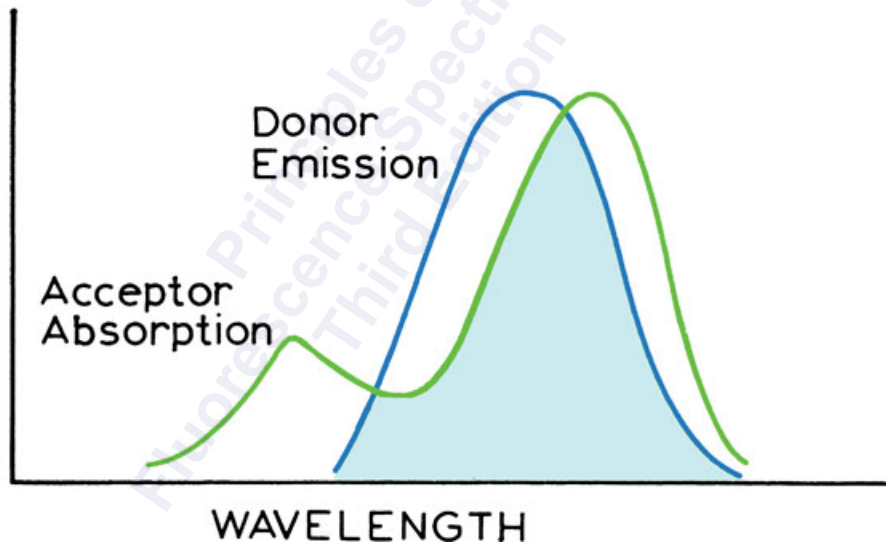
Unpolarized  
emission

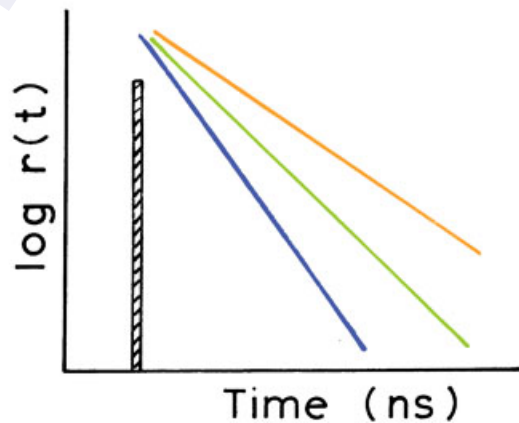
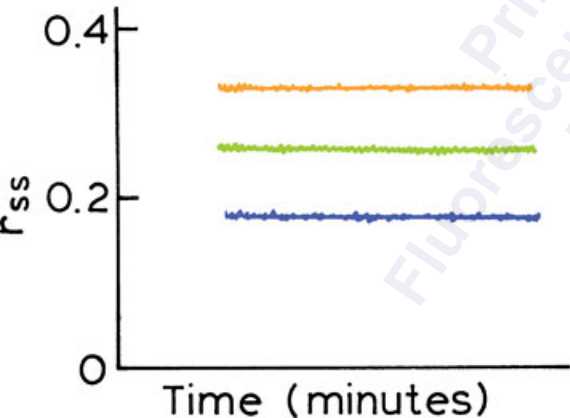
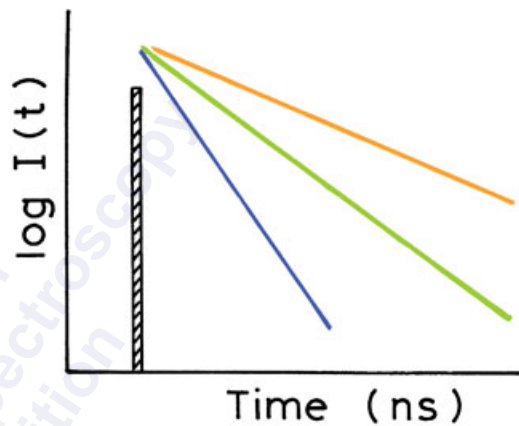
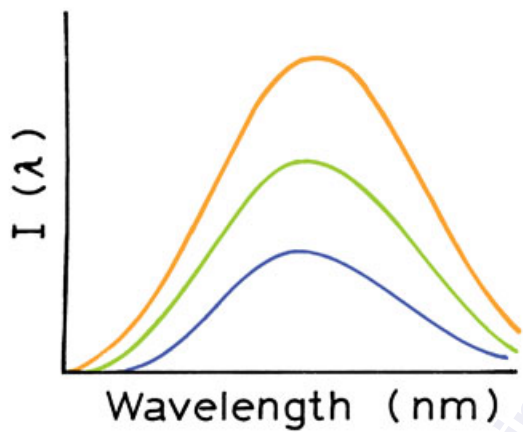


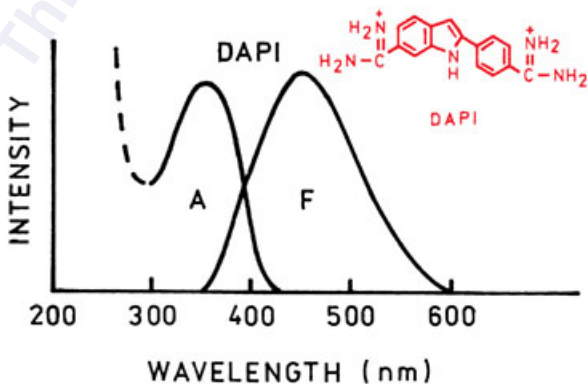
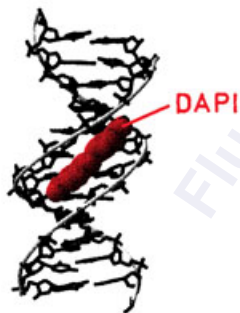
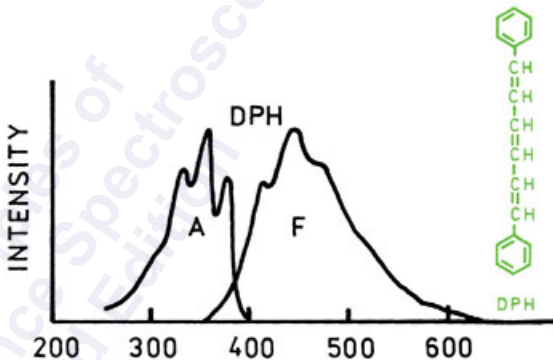
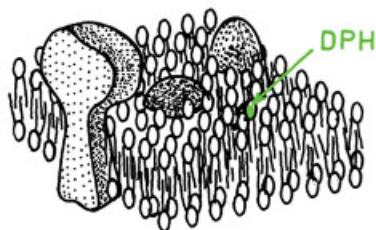
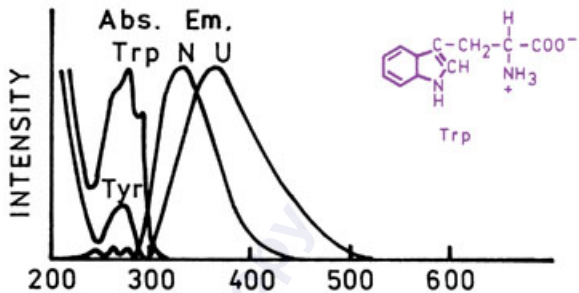
$$k_T = \frac{1}{\tau_D} \left( \frac{R_0}{r} \right)^6$$

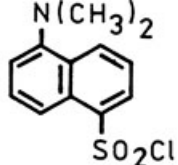
FLUORESCENCE INT.

or ABSORPTION

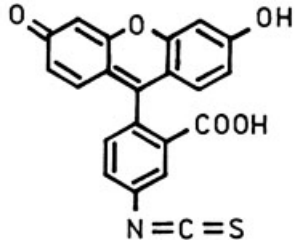




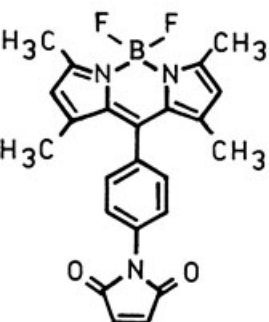




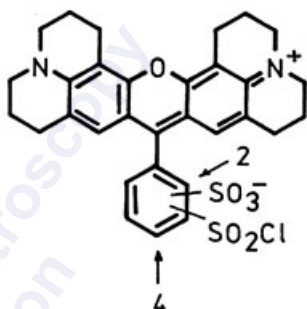
DNS-Cl



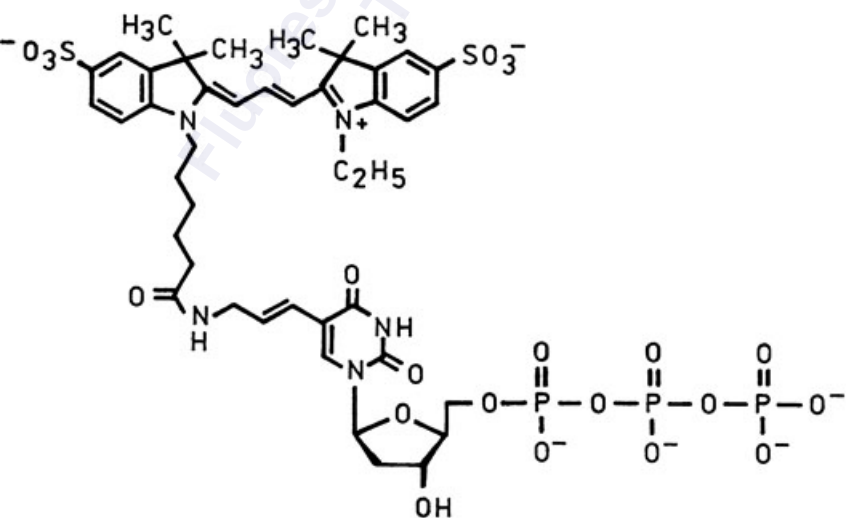
FITC



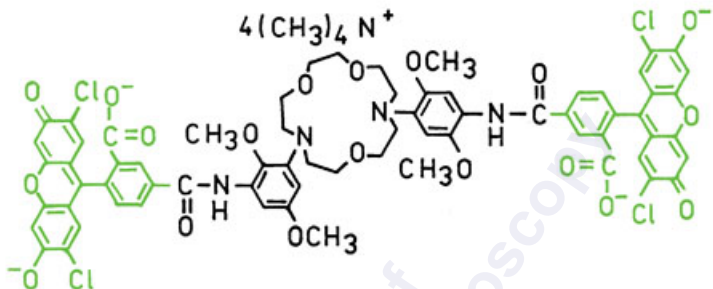
Bodipy 499/508  
maleimide



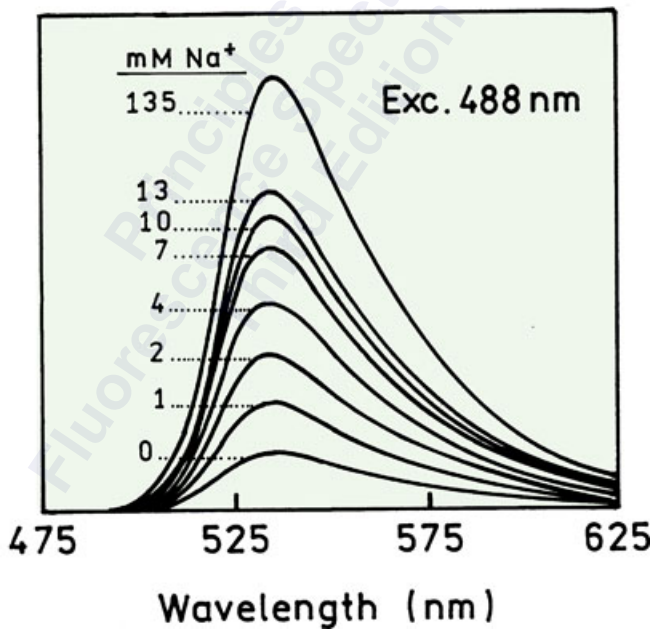
Texas Red  
sulfonyl chloride



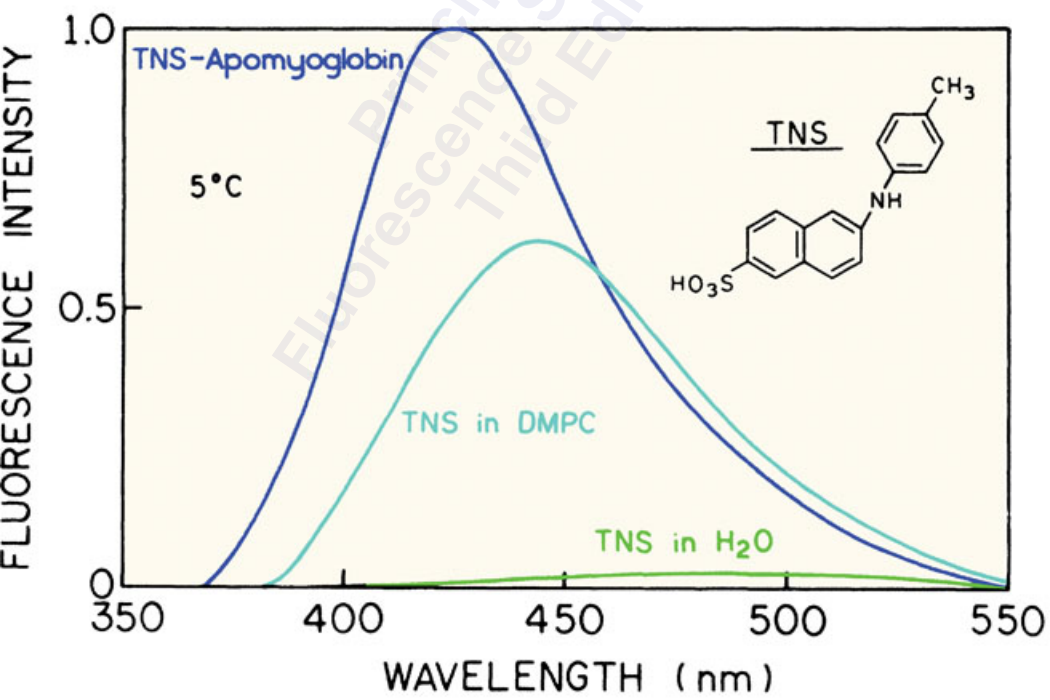
Cy3-4-dUTP

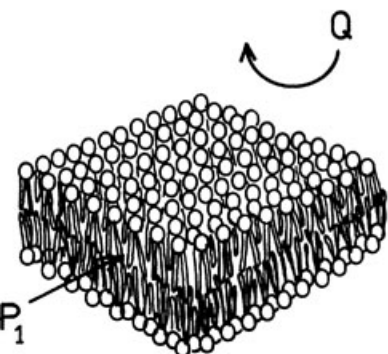
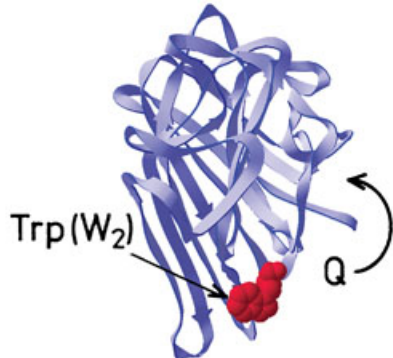
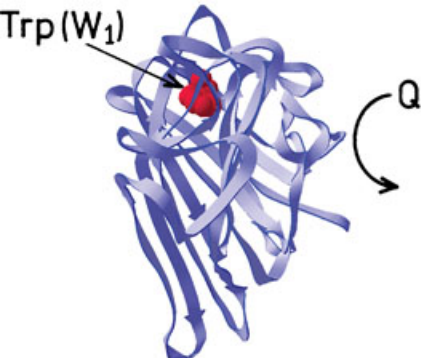


Fluorescence Emission

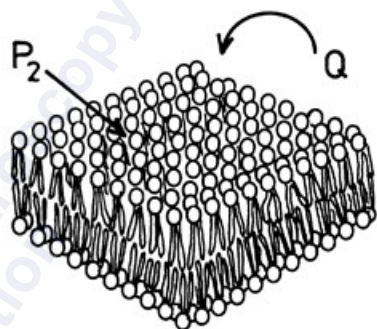




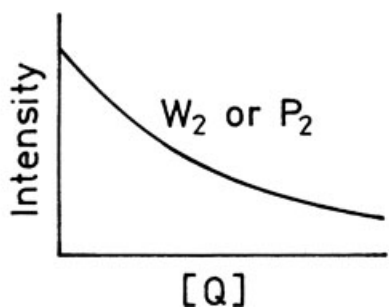
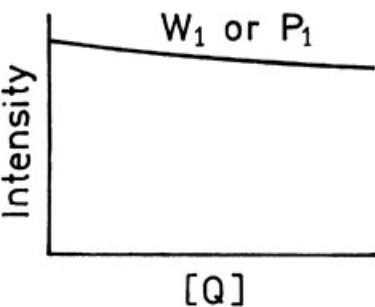
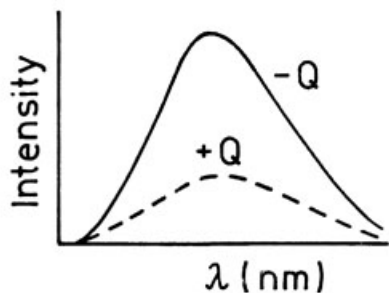
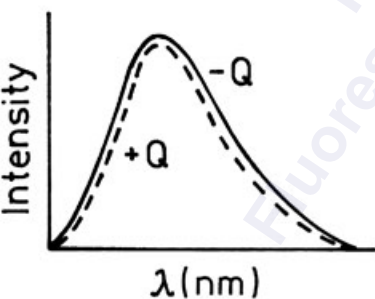


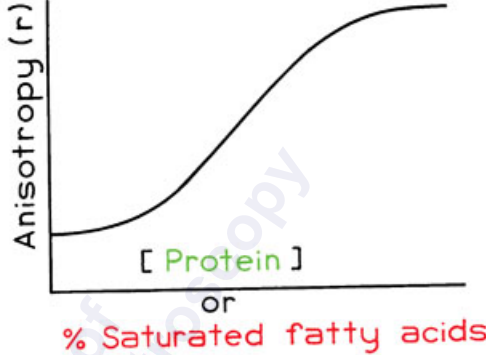
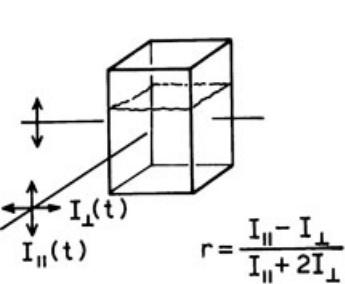


Buried Tryptophan  
or Probe



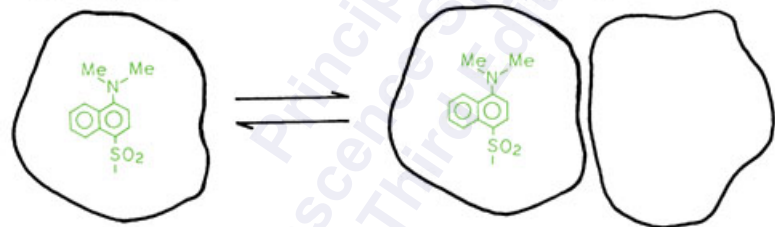
Surface Tryptophan  
or Probe





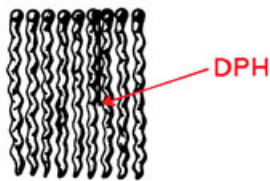
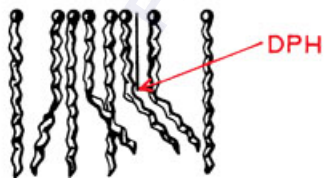
Monomer

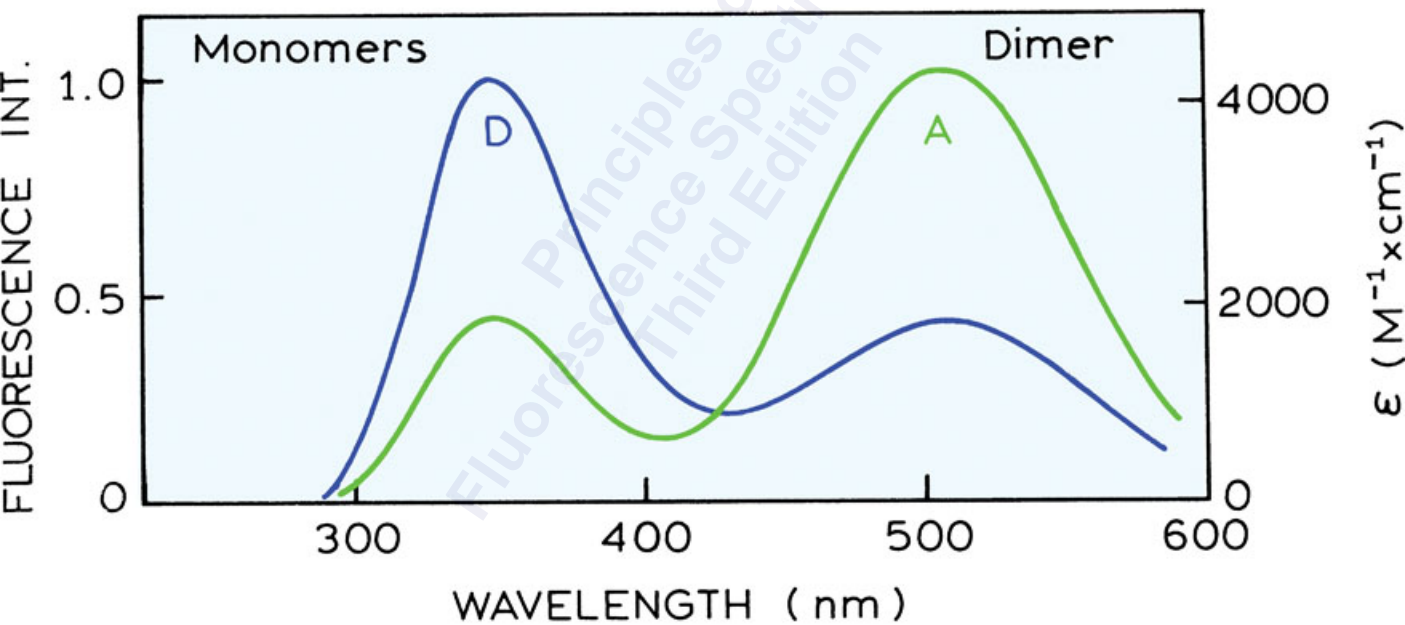
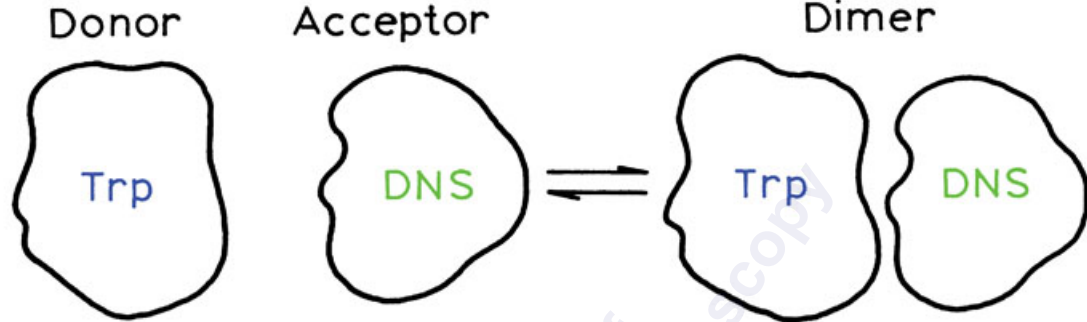
Aggregate

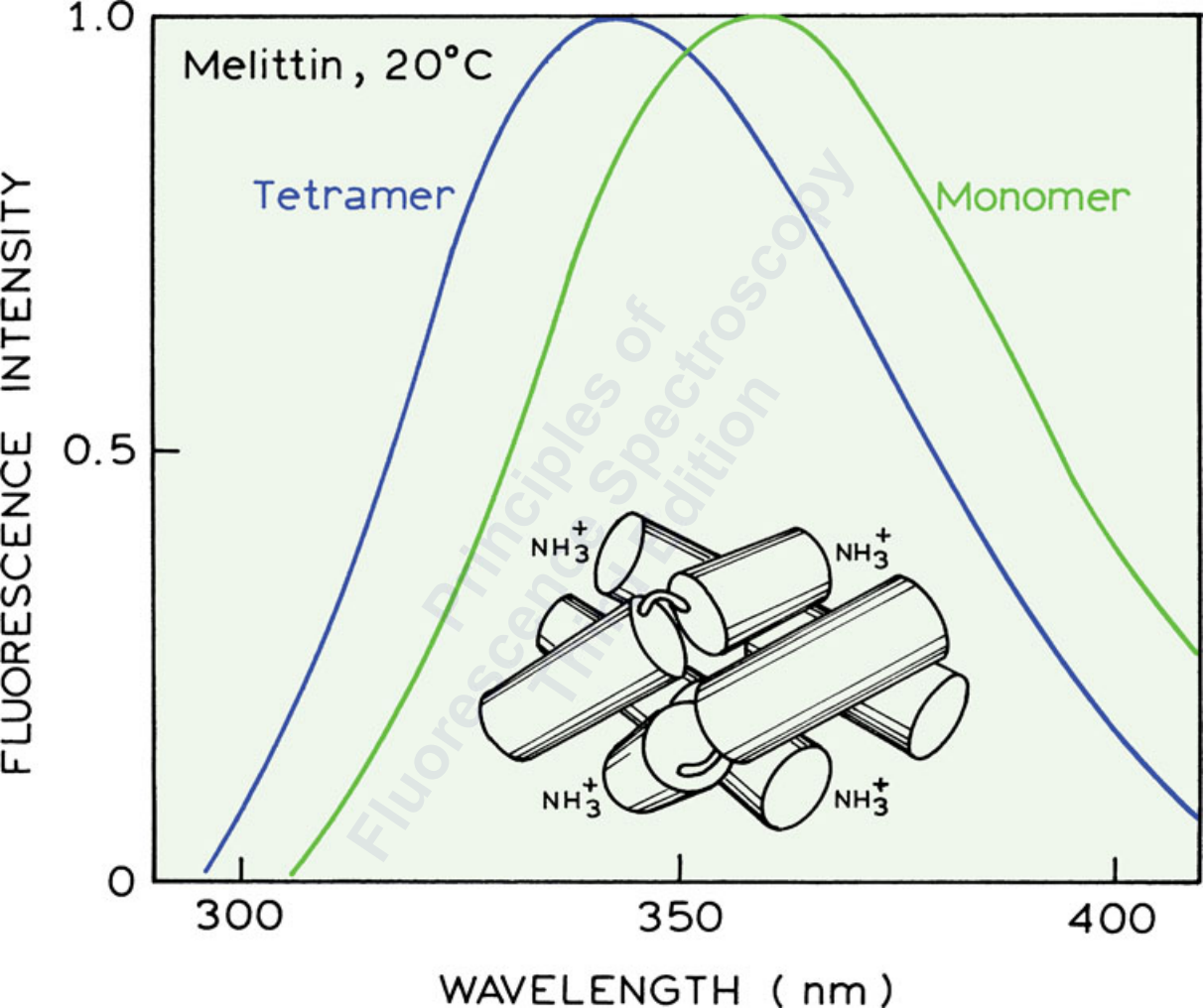


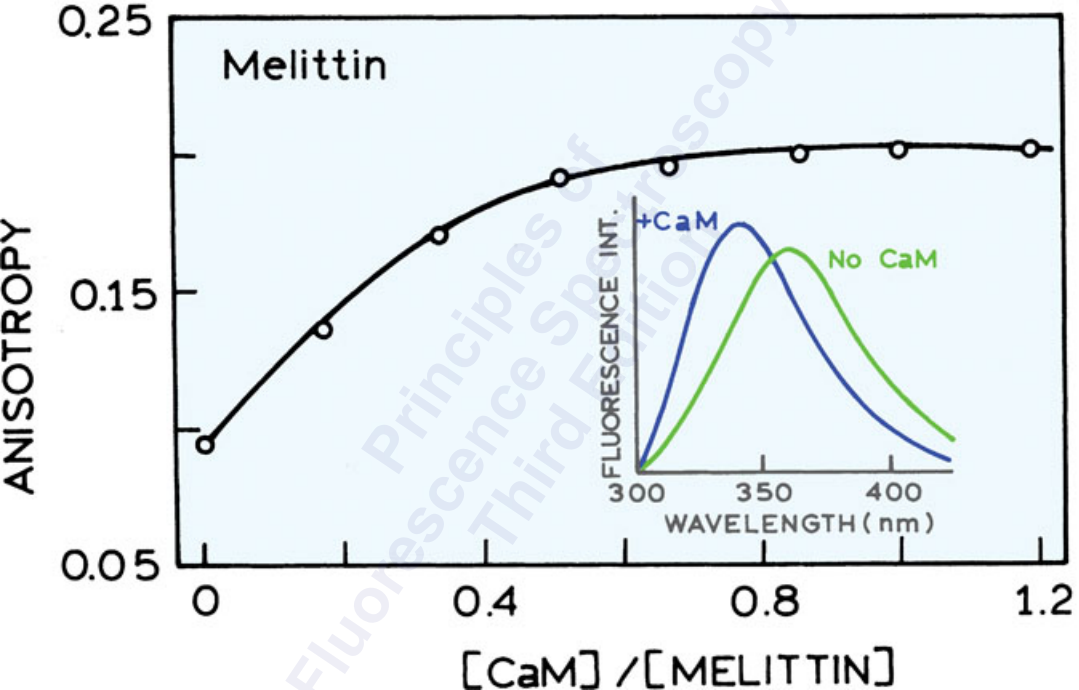
Unsaturated Lipids

Saturated Lipids

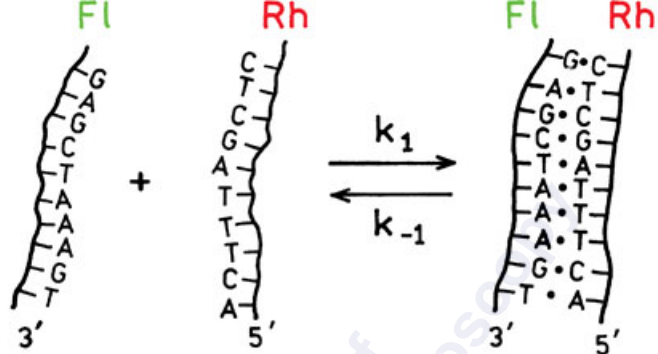




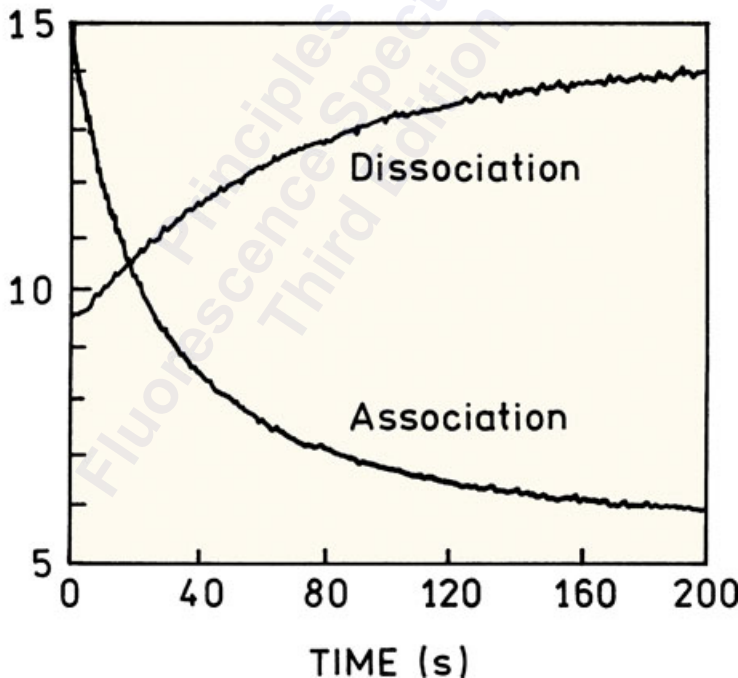


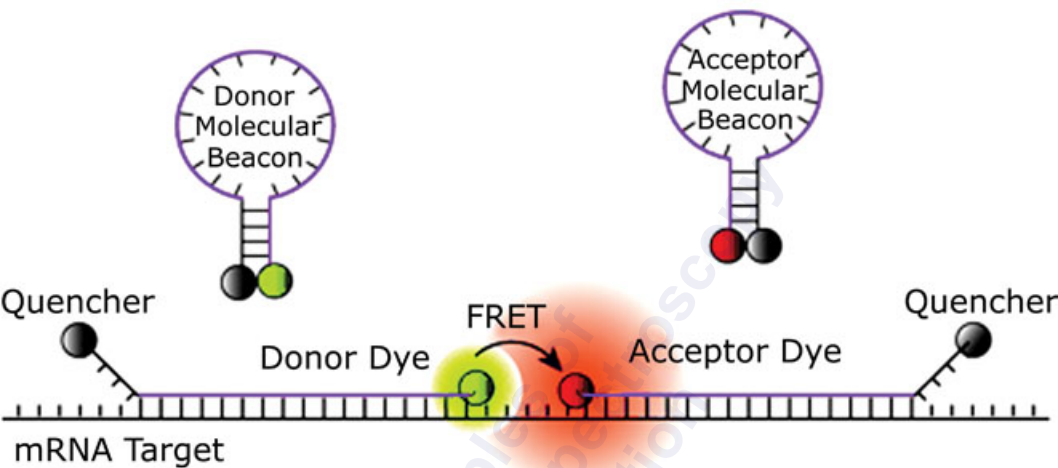






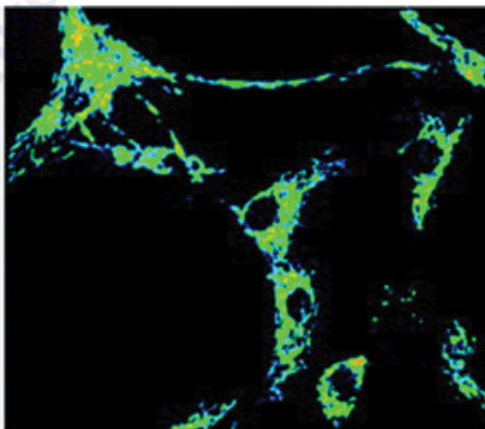
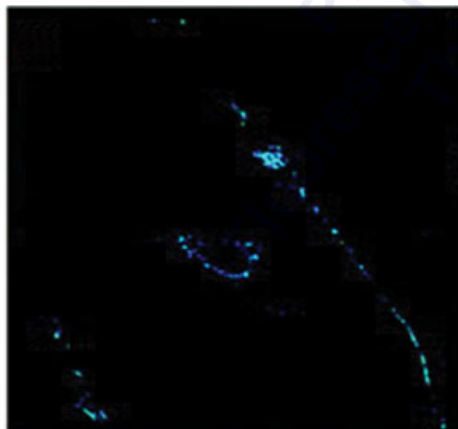
DONOR FLUORESCENCE INTENSITY

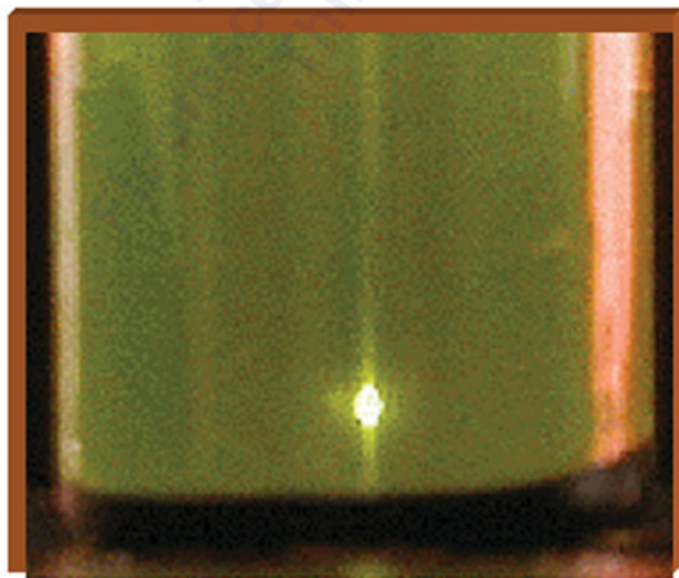
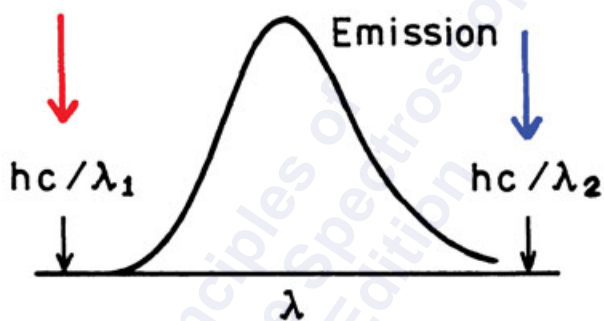
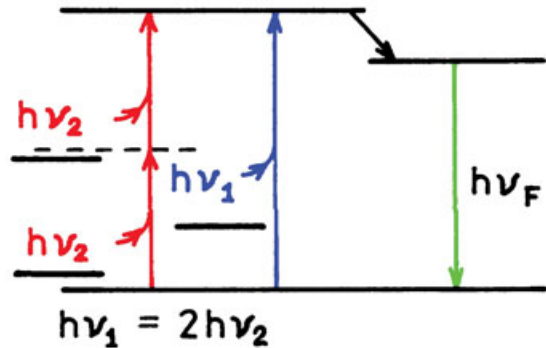


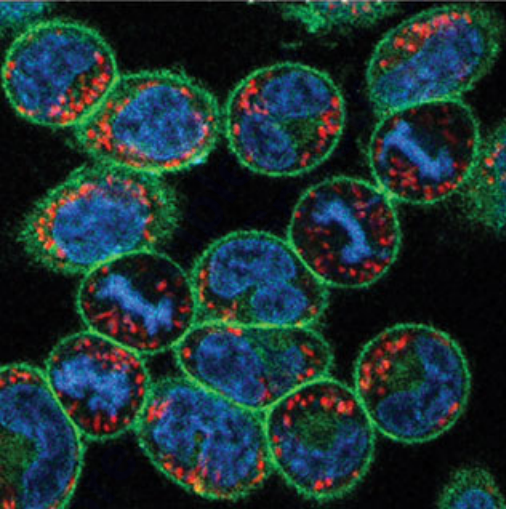


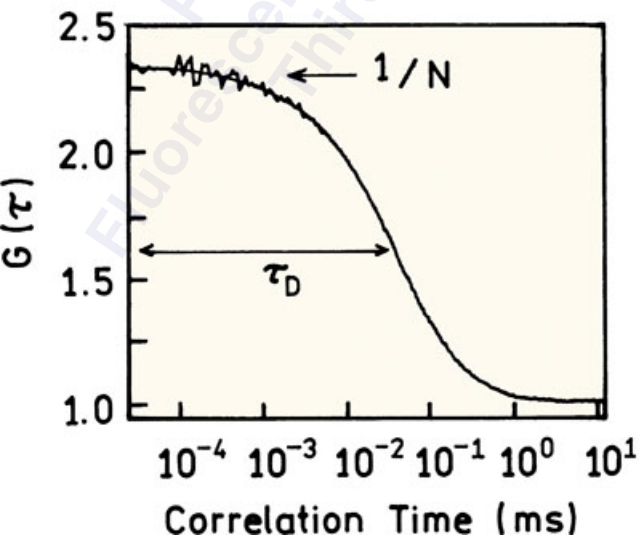
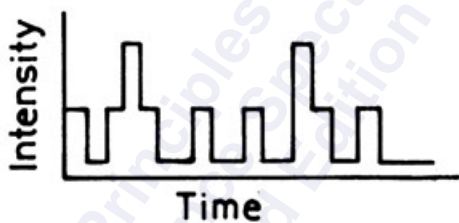
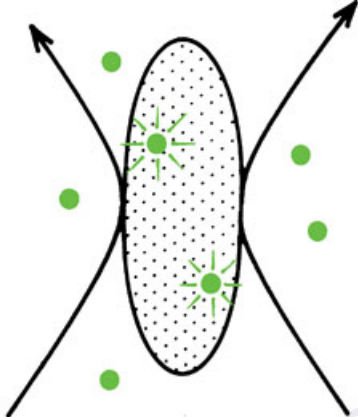
Control

Stimulated



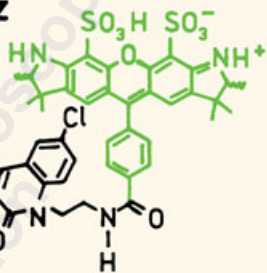






$G(\tau)$ , normalized

Alexa-Bz



Receptor  
 $\tau_D$  (ms)

-  
7

+  
362

$\tau$  (ms)

1.0

0.5

0

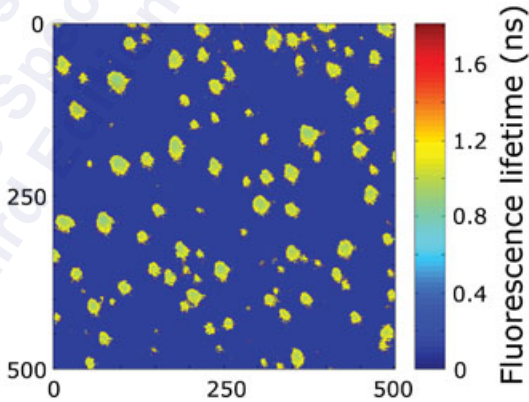
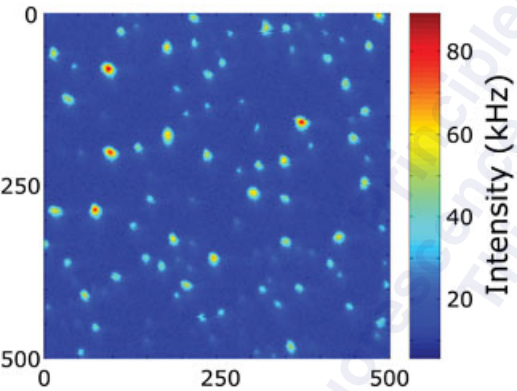
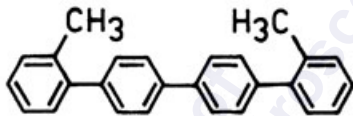
$10^{-3}$

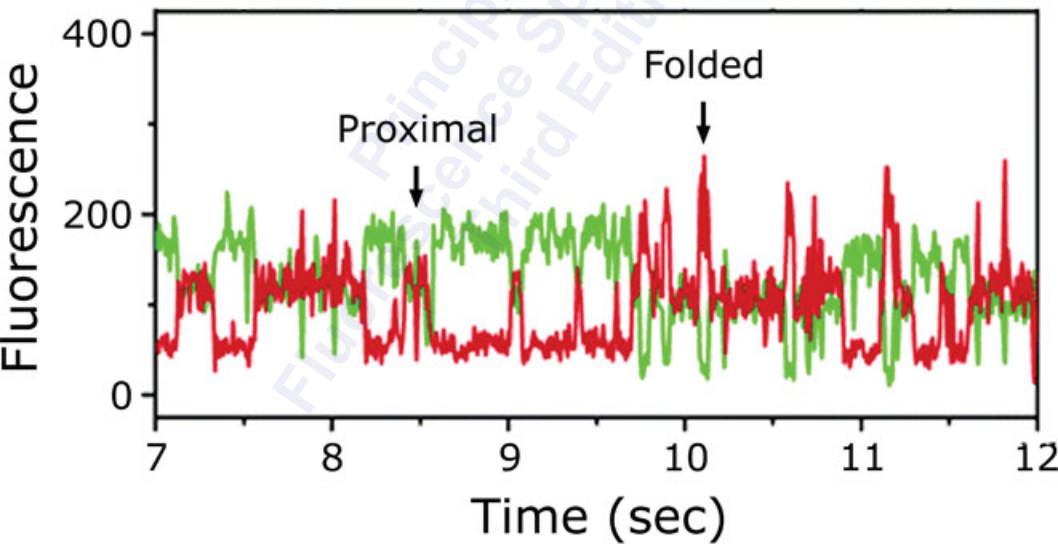
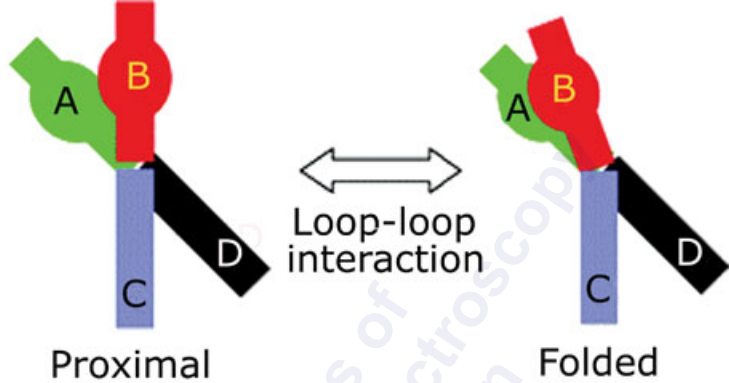
$10^{-1}$

$10^1$

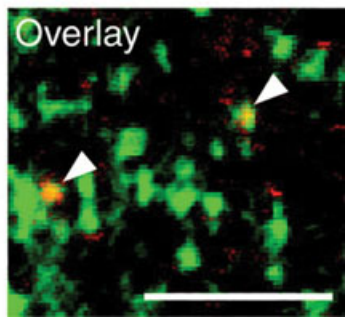
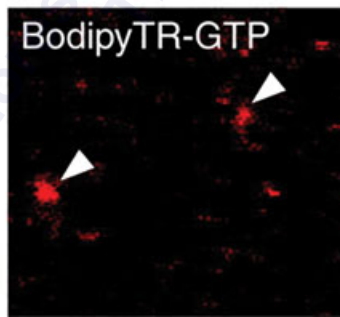
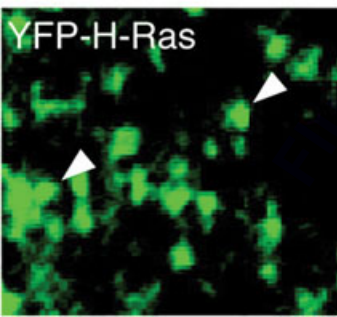
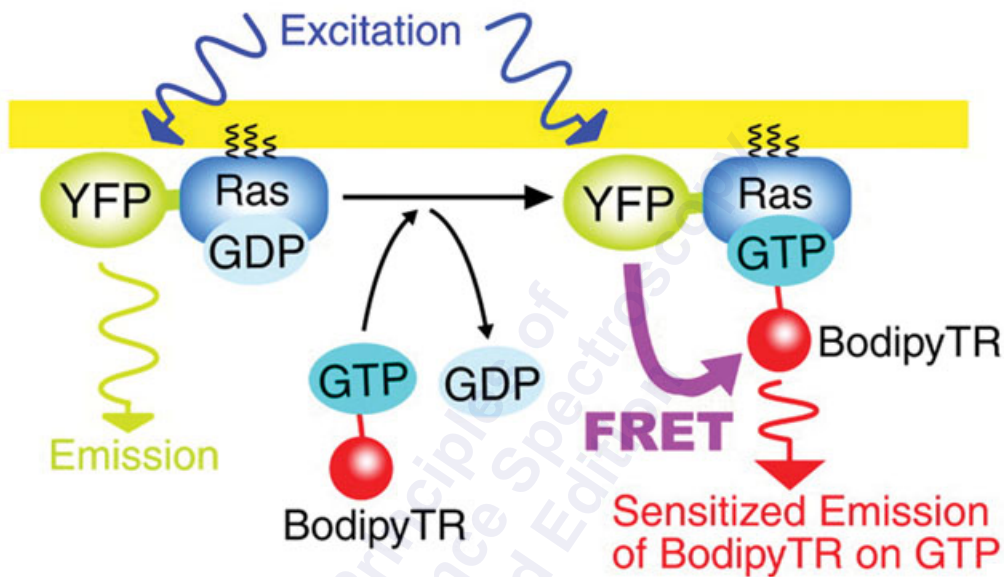
$10^3$

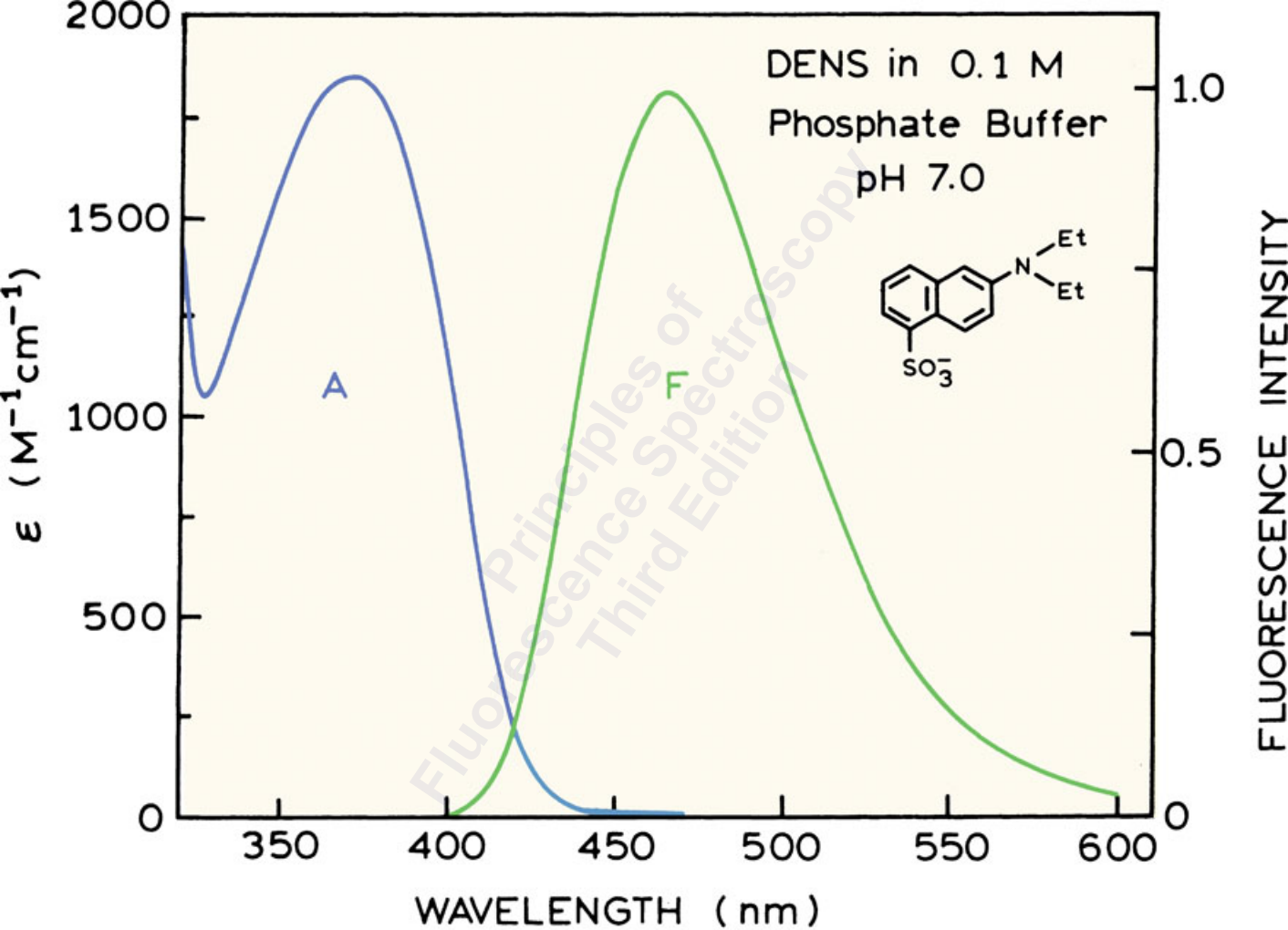
$10^5$



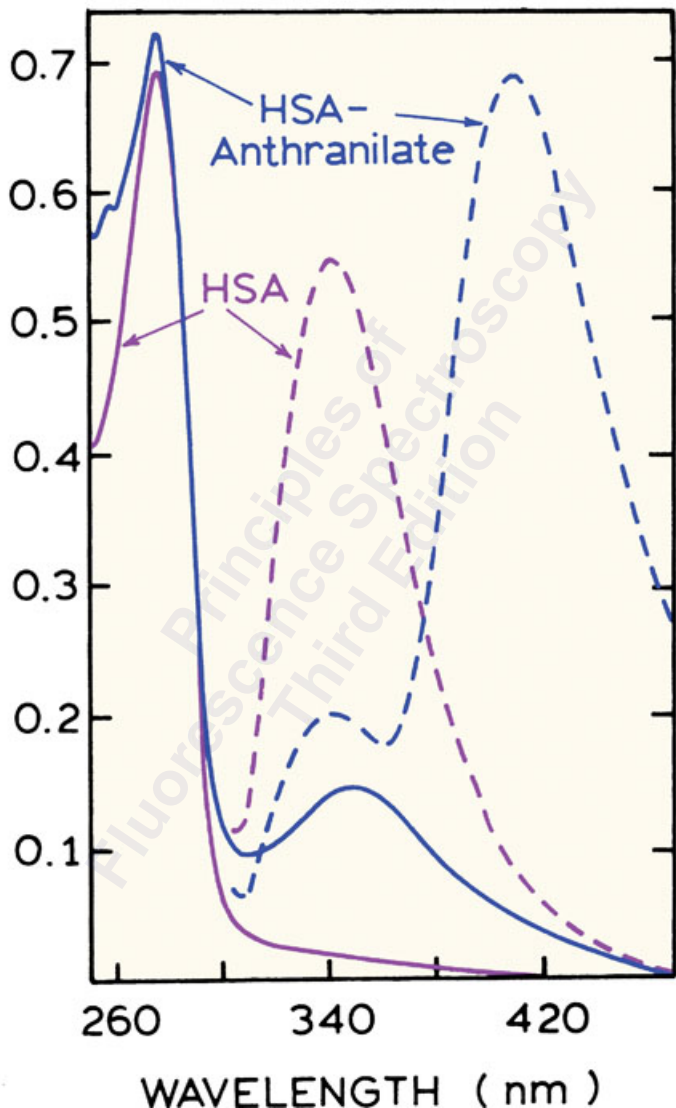




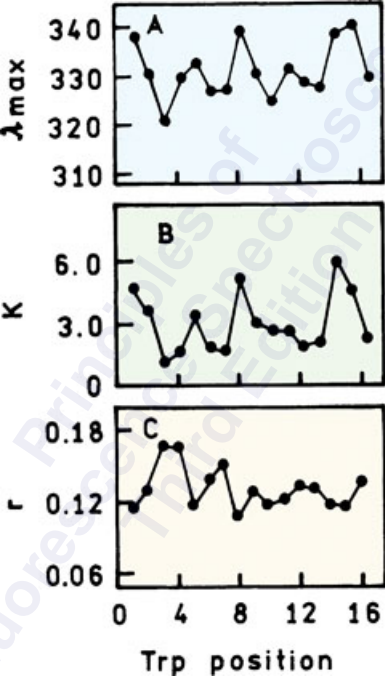




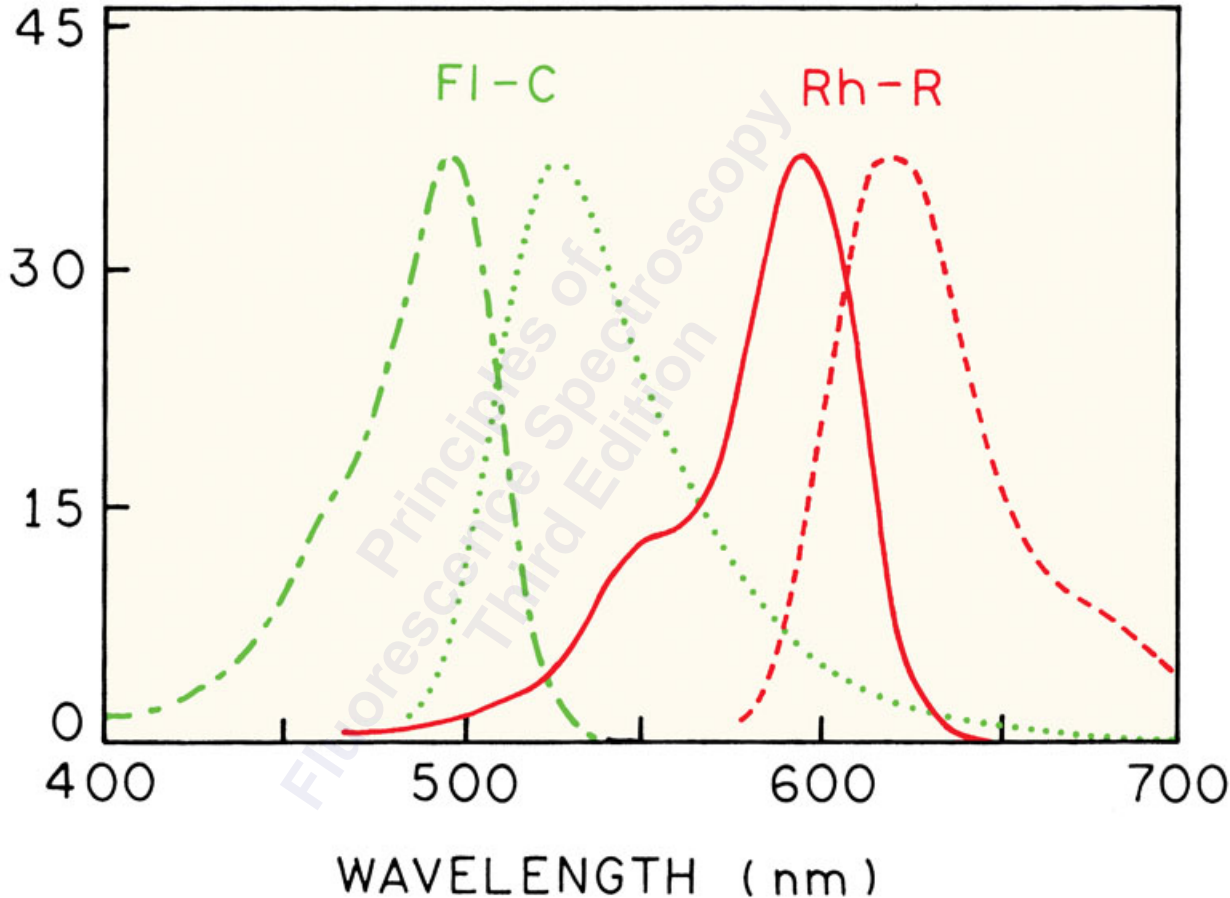
ABSORBANCE (—)

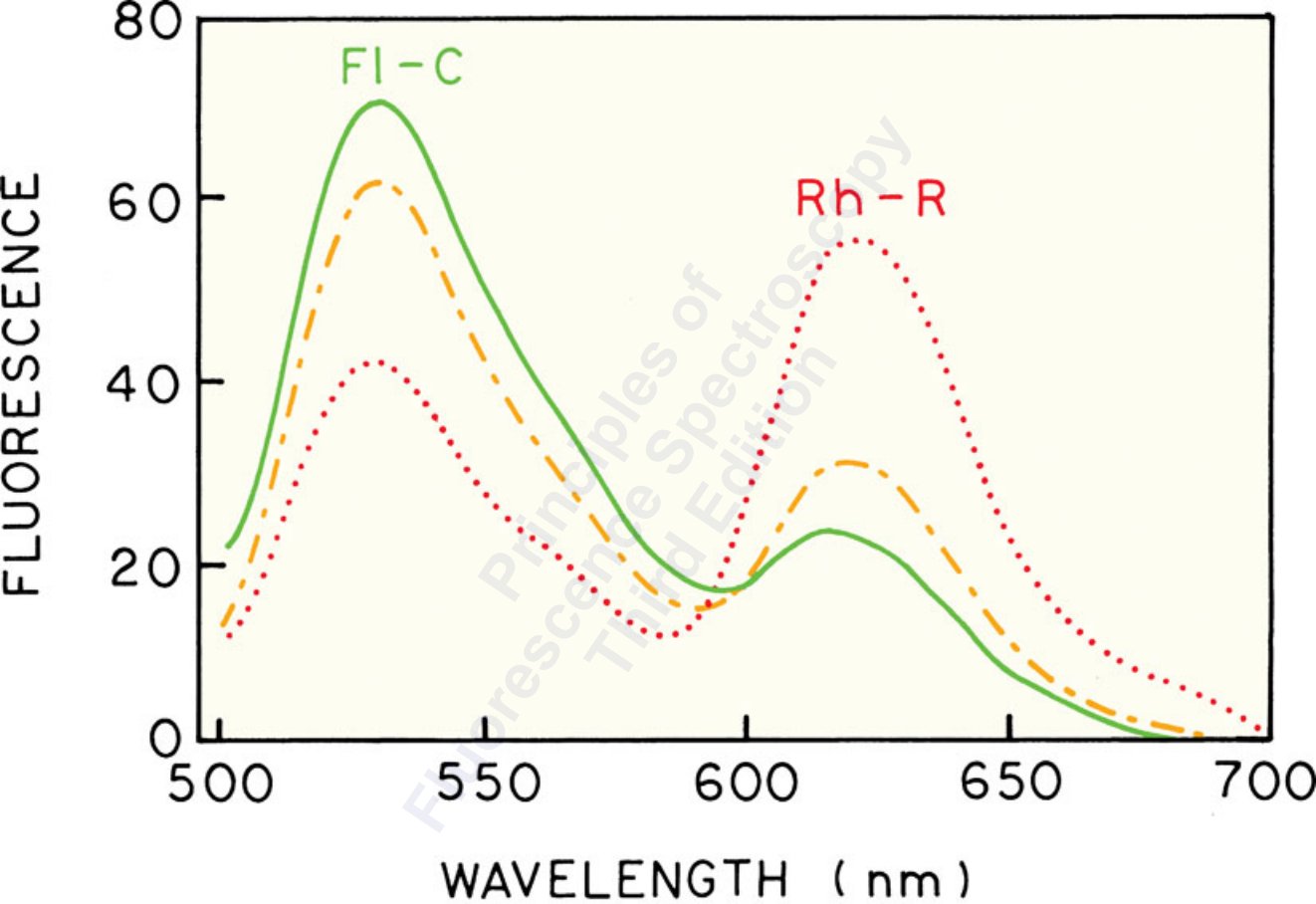


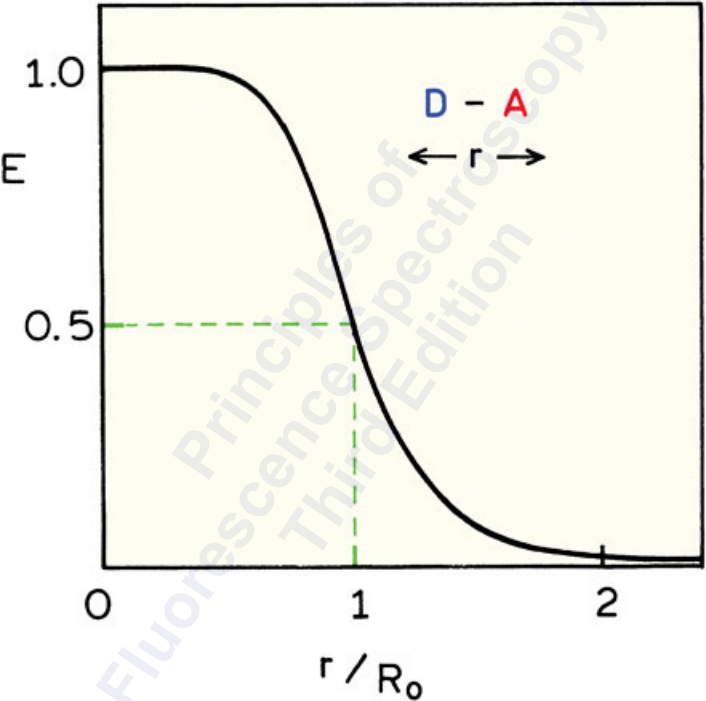
RELATIVE FLUORESCENCE (---)

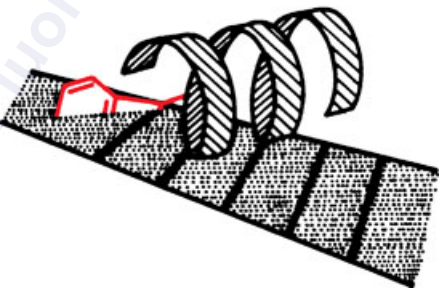
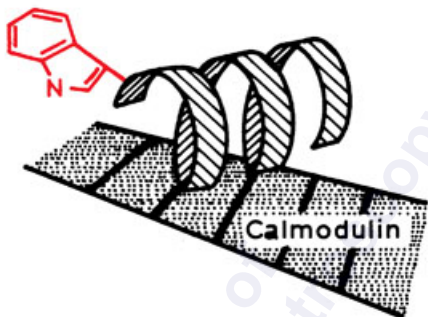


ABSORPTION or  
FLUORESCENCE

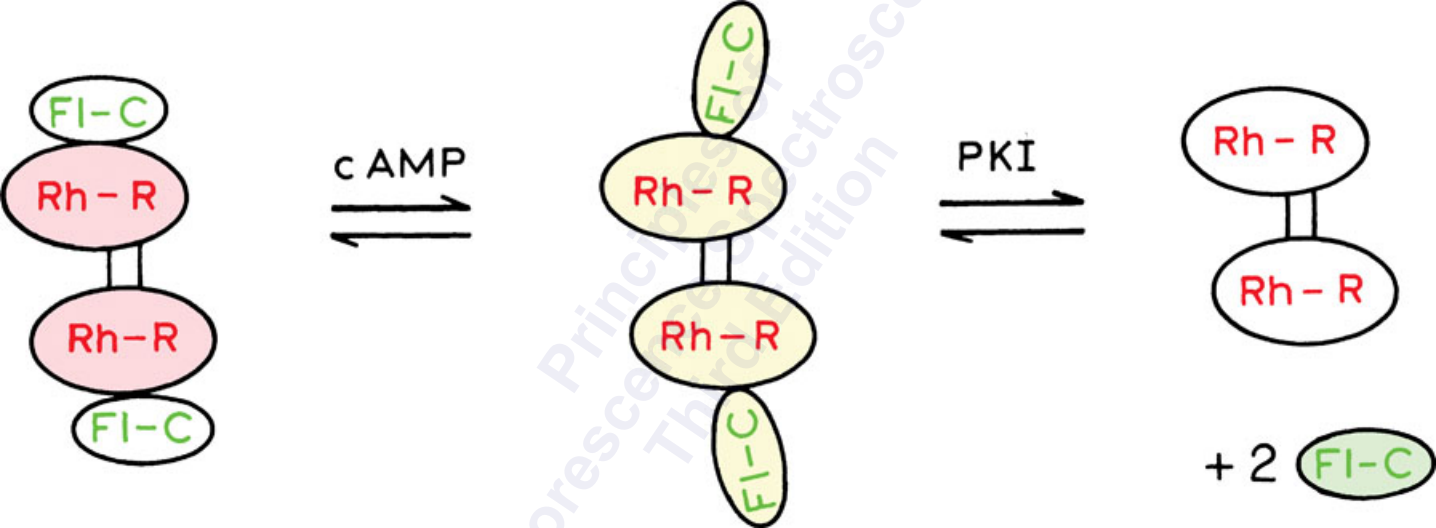


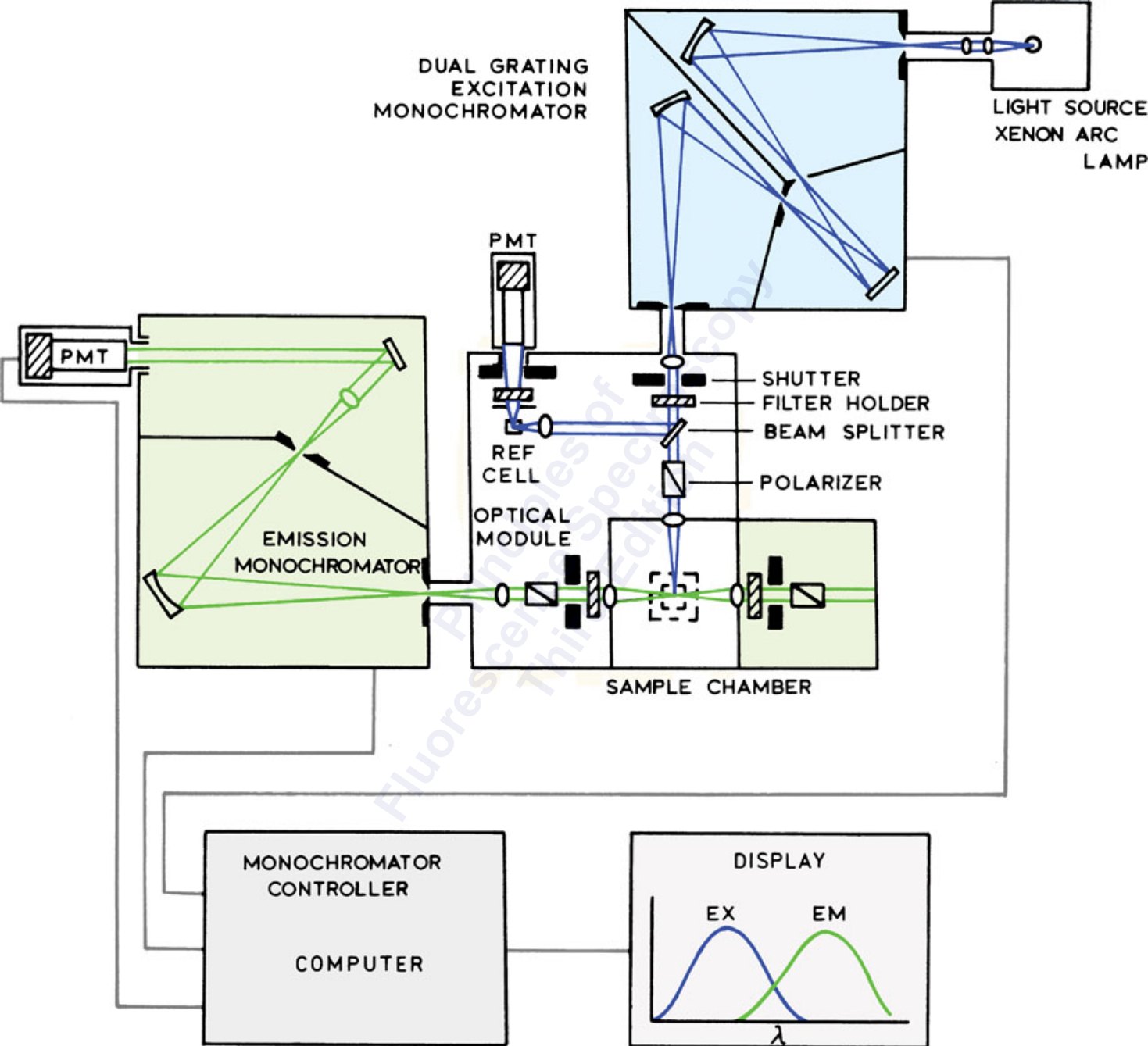


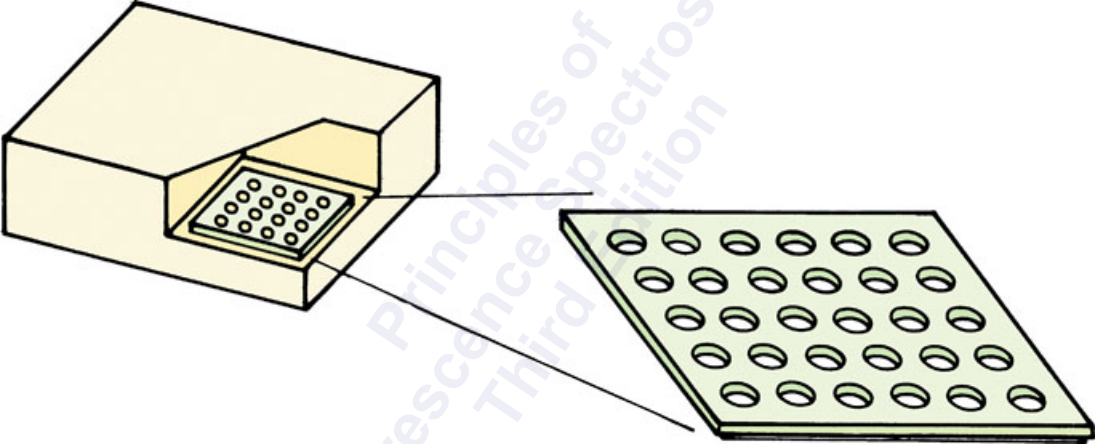


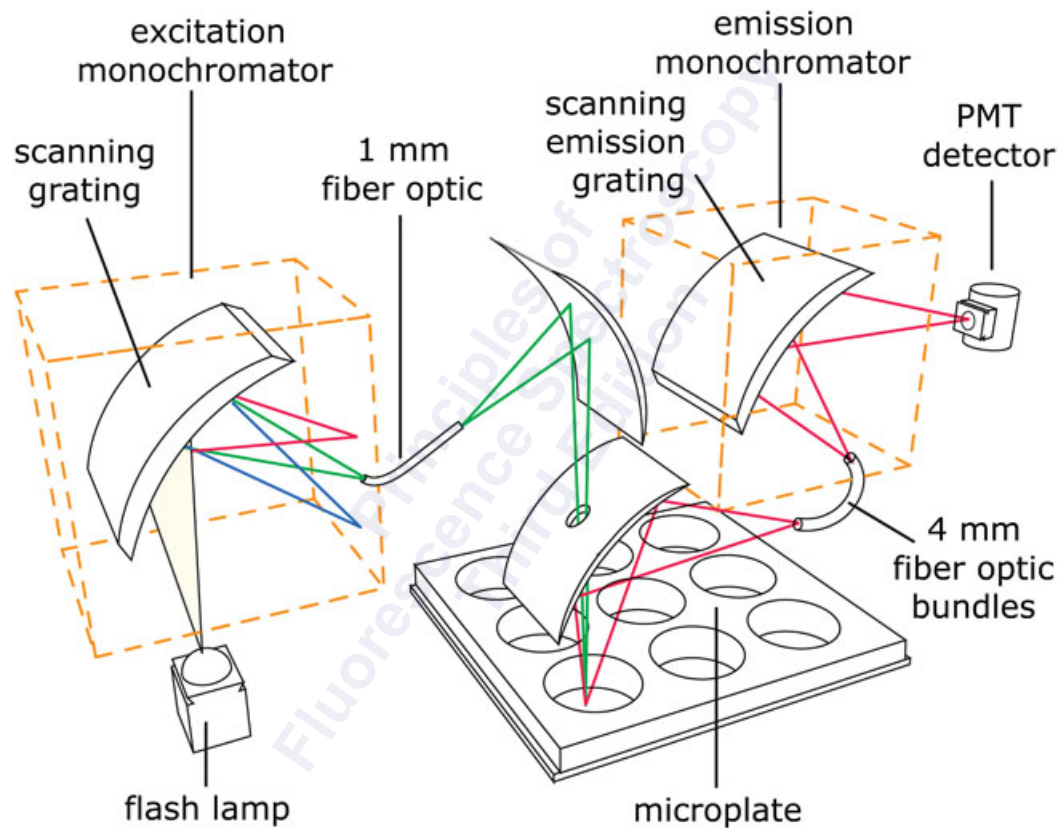








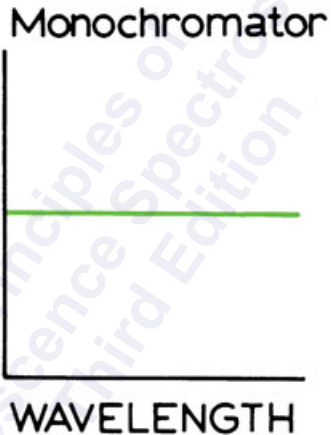




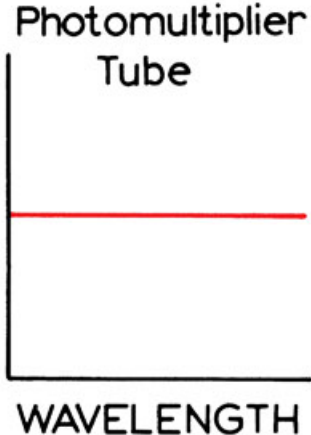
LIGHT INTENSITY



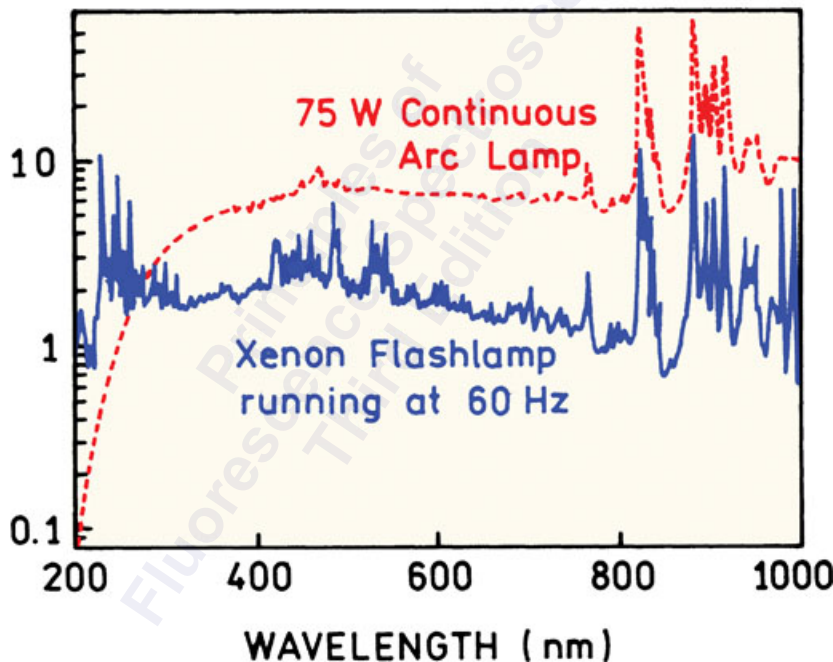
EFFICIENCY

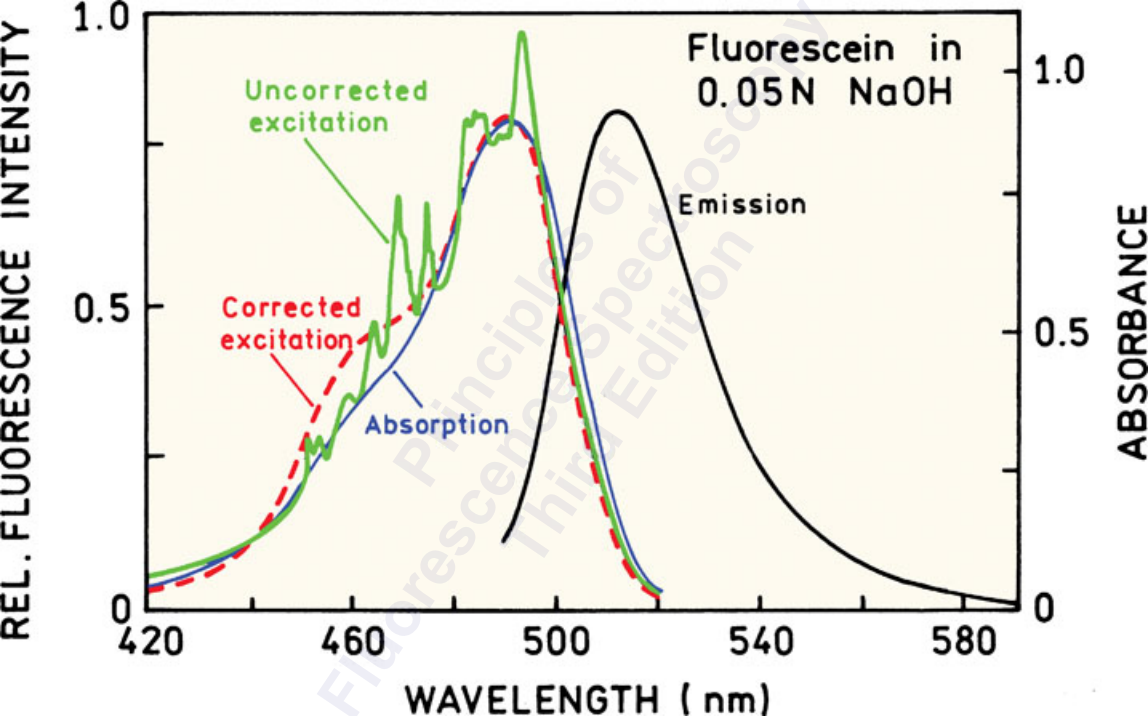


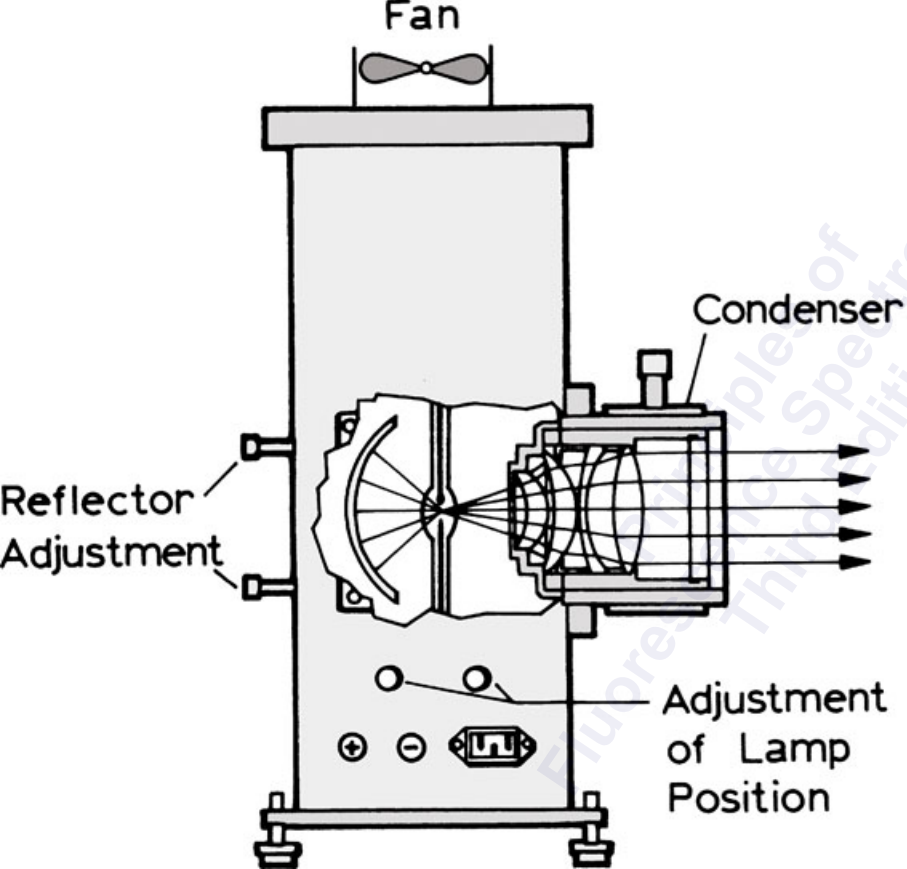
EFFICIENCY



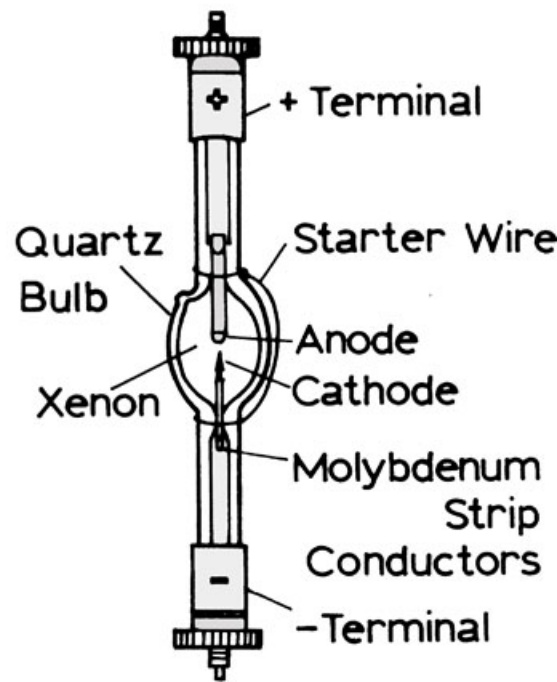
IRRADIANCE at 0.5m ( $\text{mW m}^{-2} \text{nm}^{-1}$ )



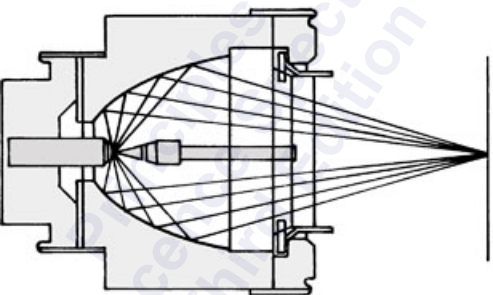
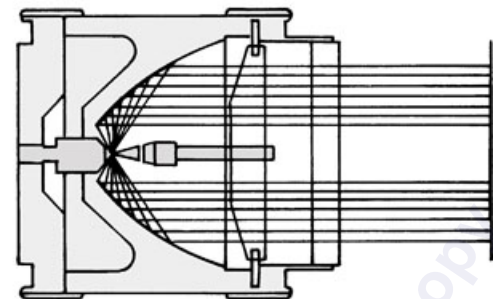




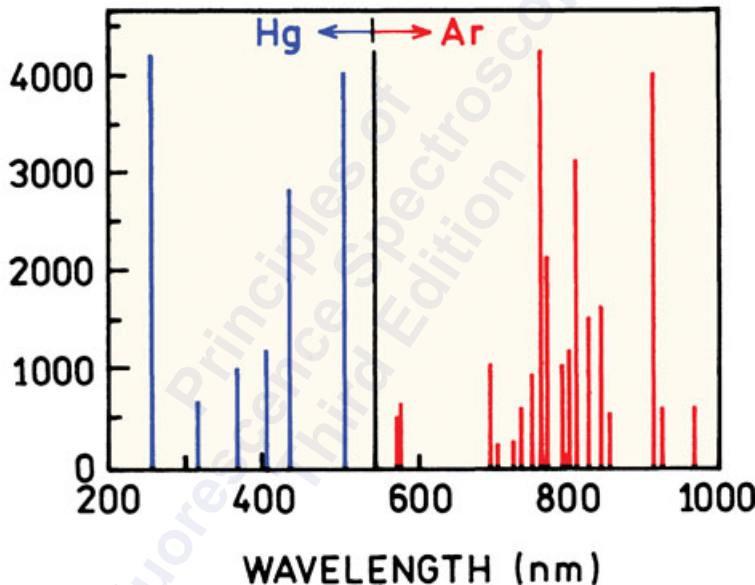
Xenon Arc Lamp



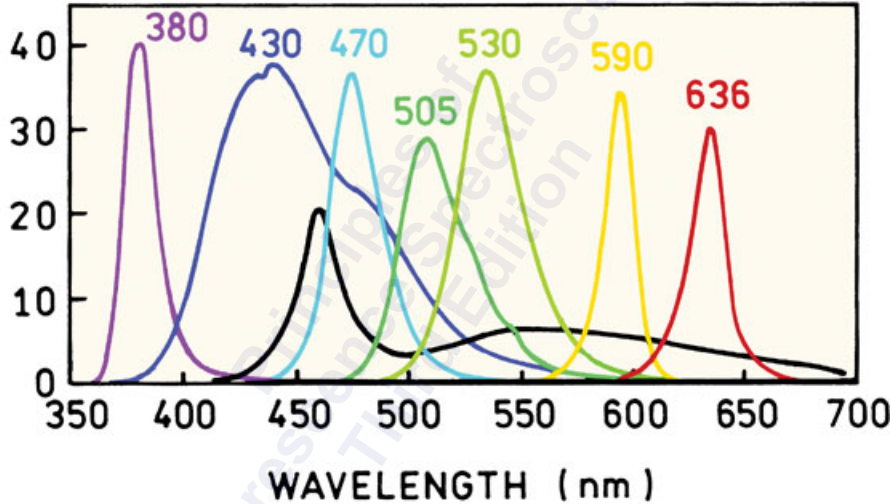




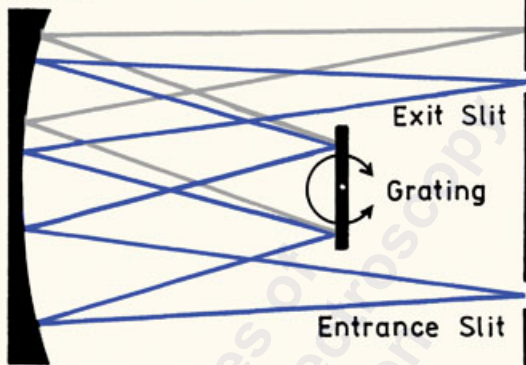
INTENSITY



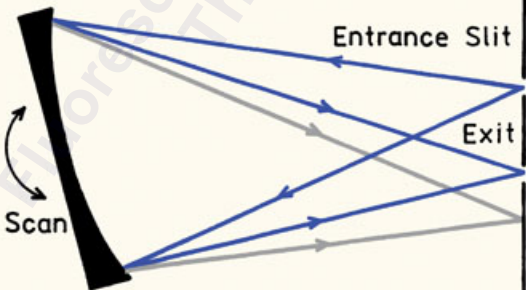
INTENSITY



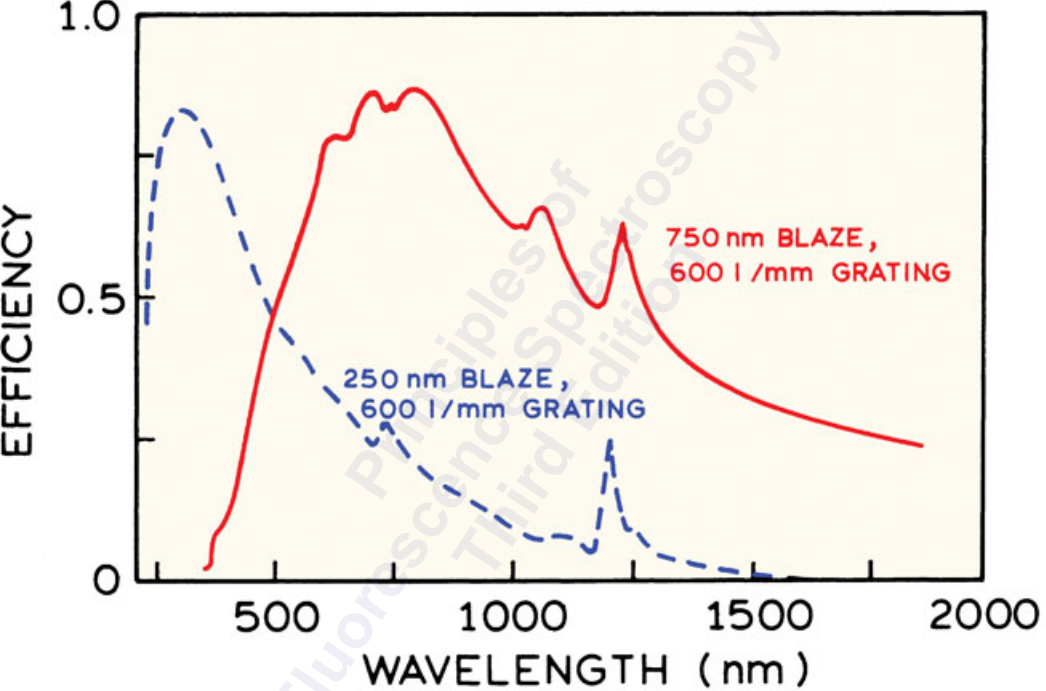
Collimating  
Mirror



Plane Grating



Concave Grating



FLUORESCENCE INTENSITY

1.0

0.5

300

350

400

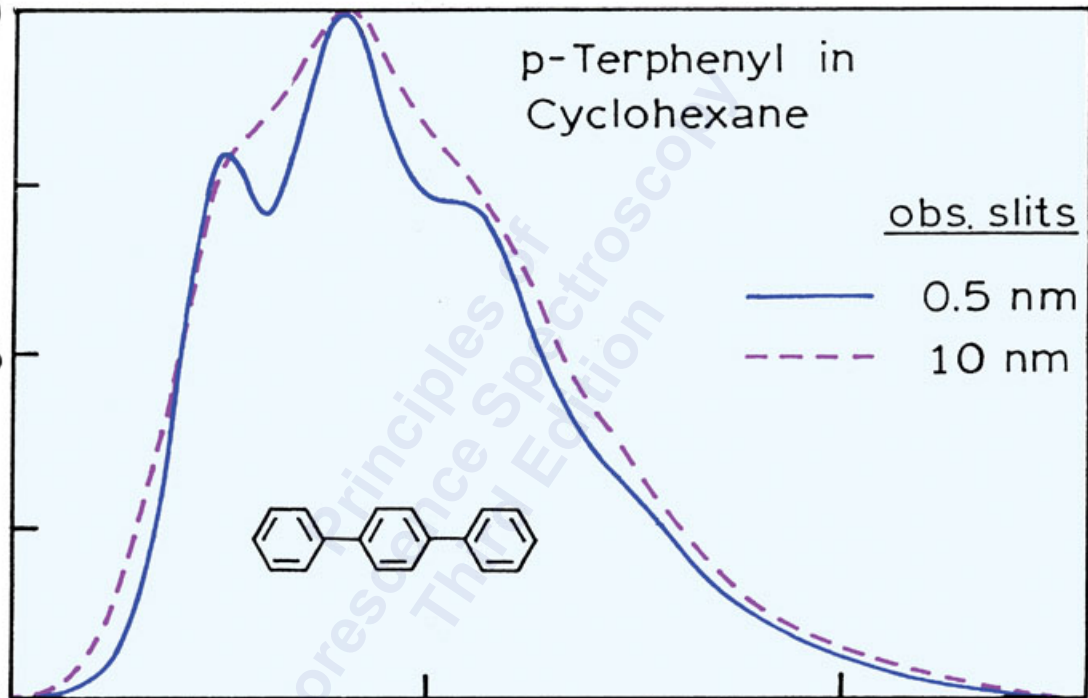
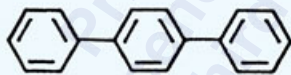
WAVELENGTH ( nm )

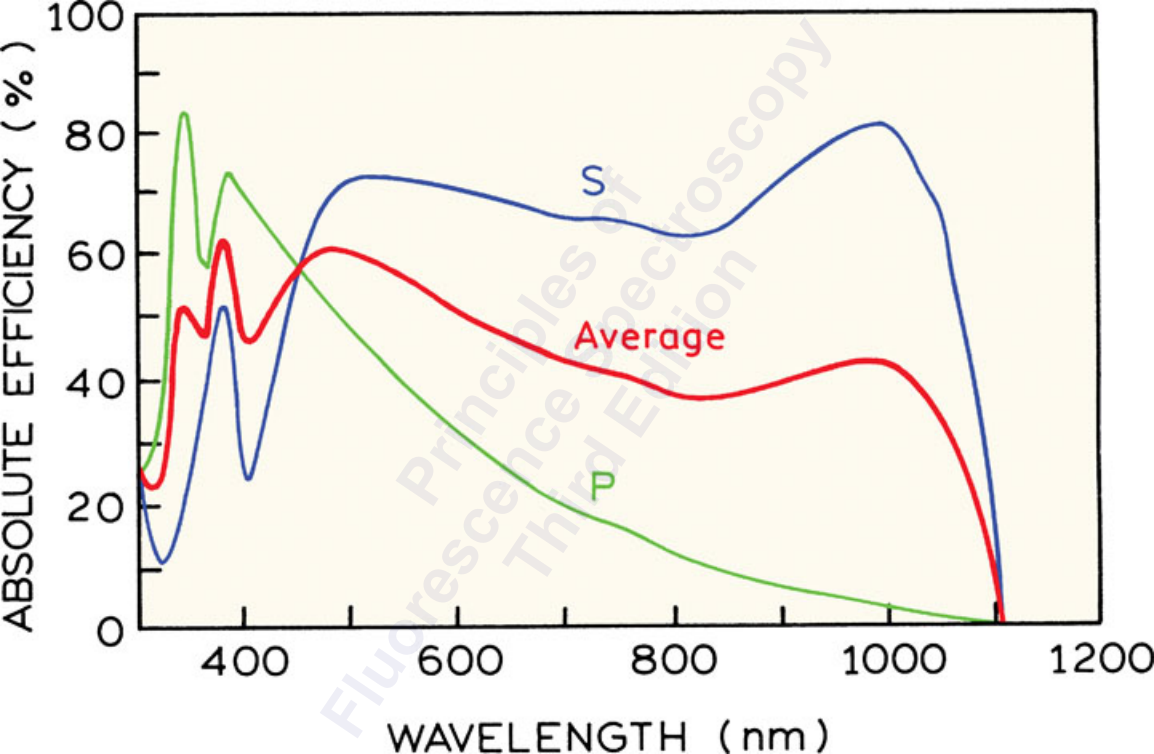
p-Terphenyl in  
Cyclohexane

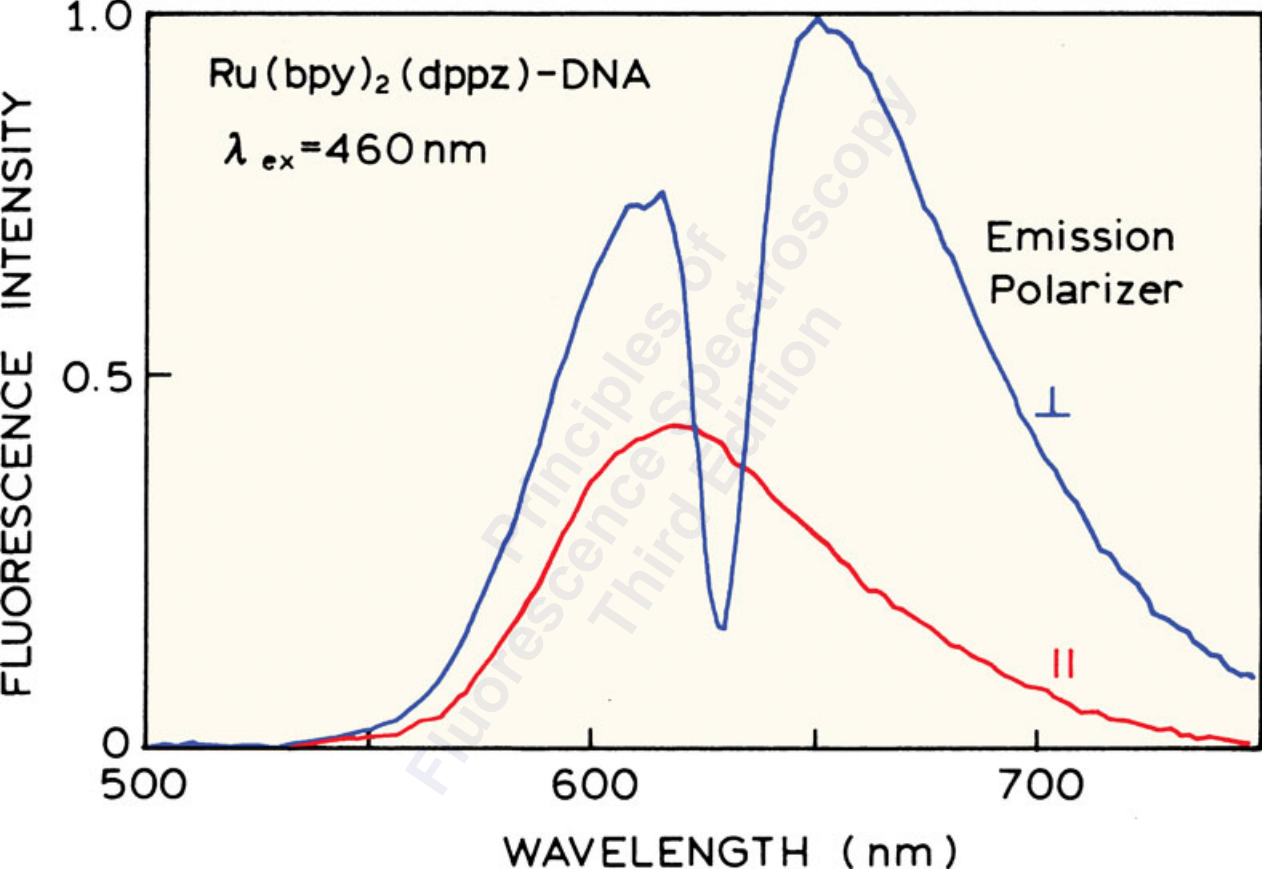
obs. slits

0.5 nm

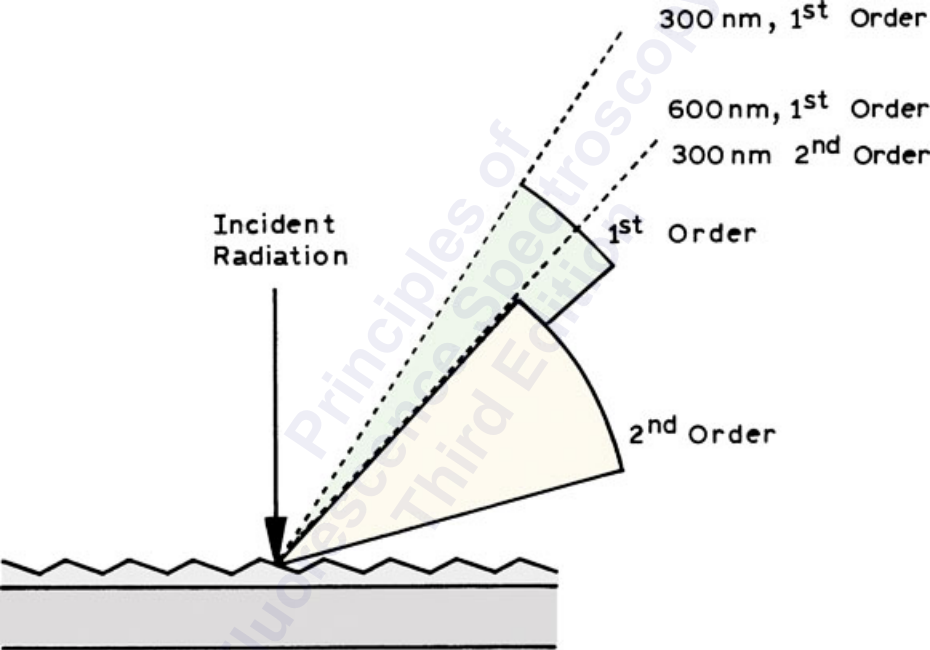
10 nm



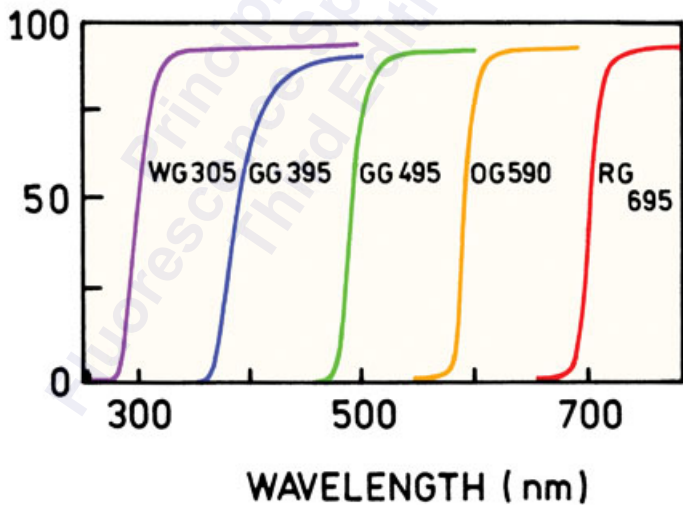
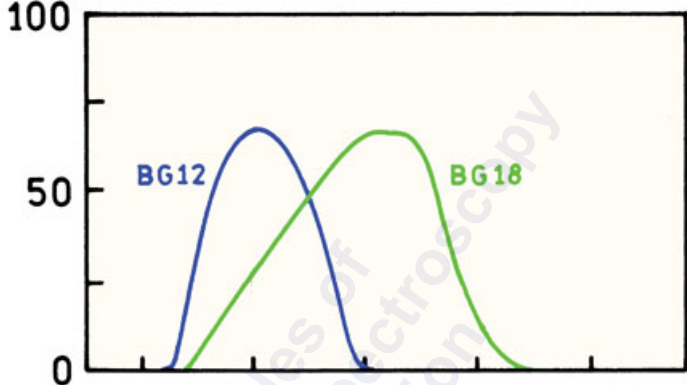




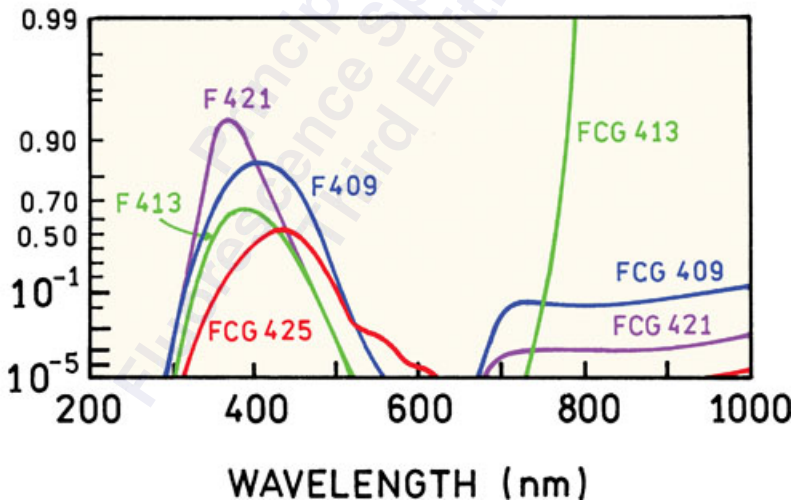
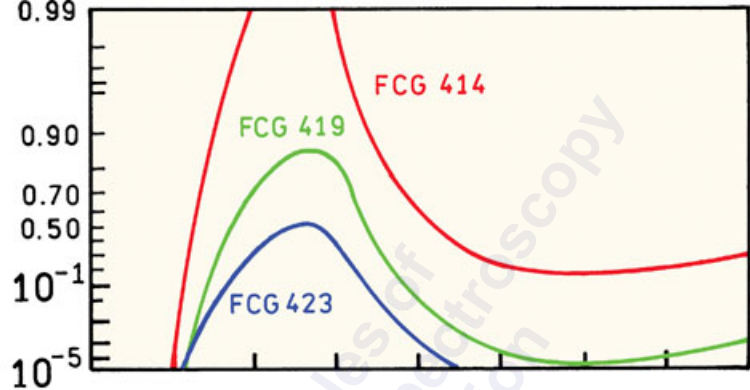




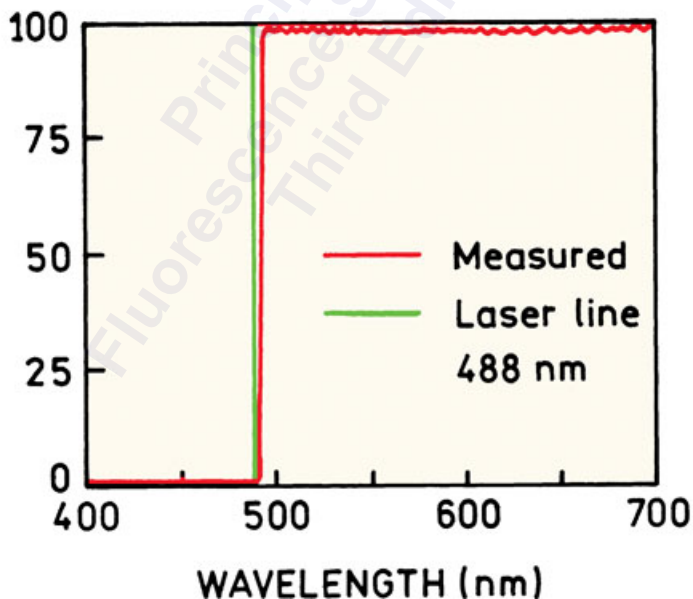
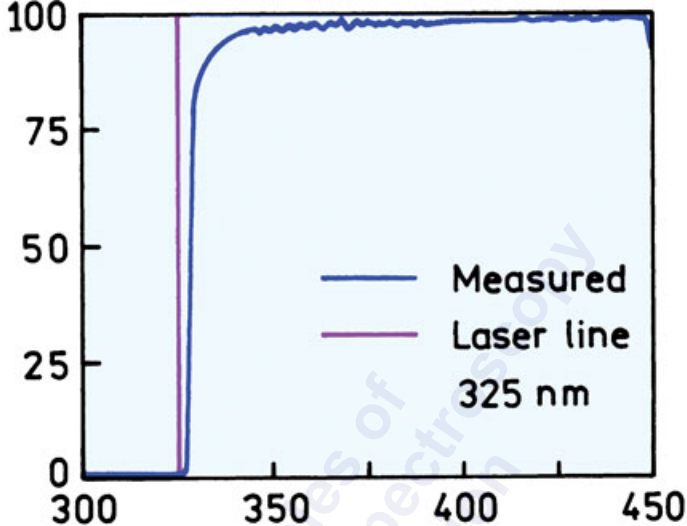
FRACTIONAL TRANSMITTANCE

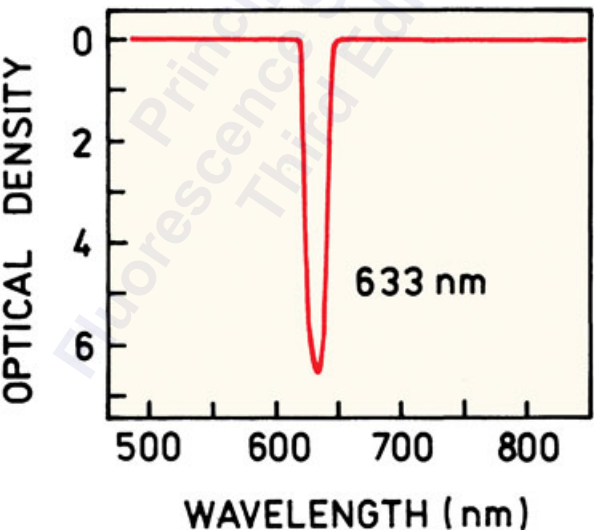
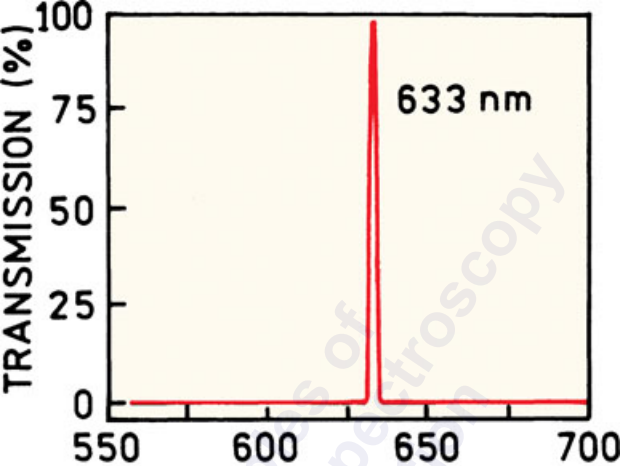


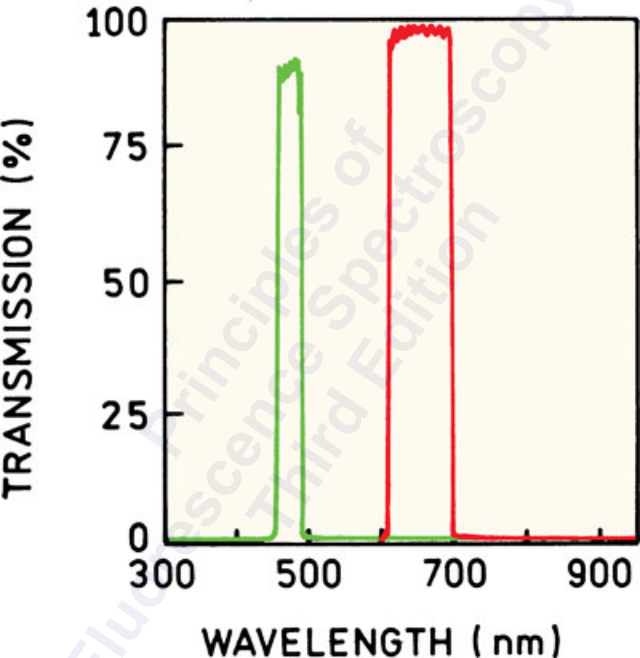
FRACTIONAL TRANSMITTANCE



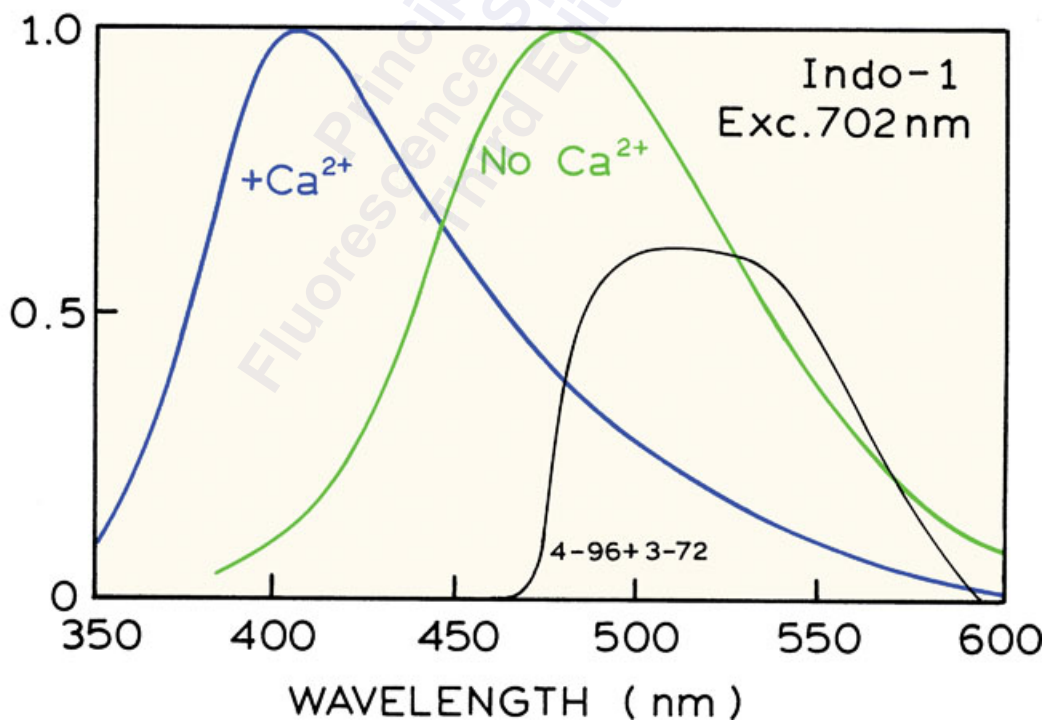
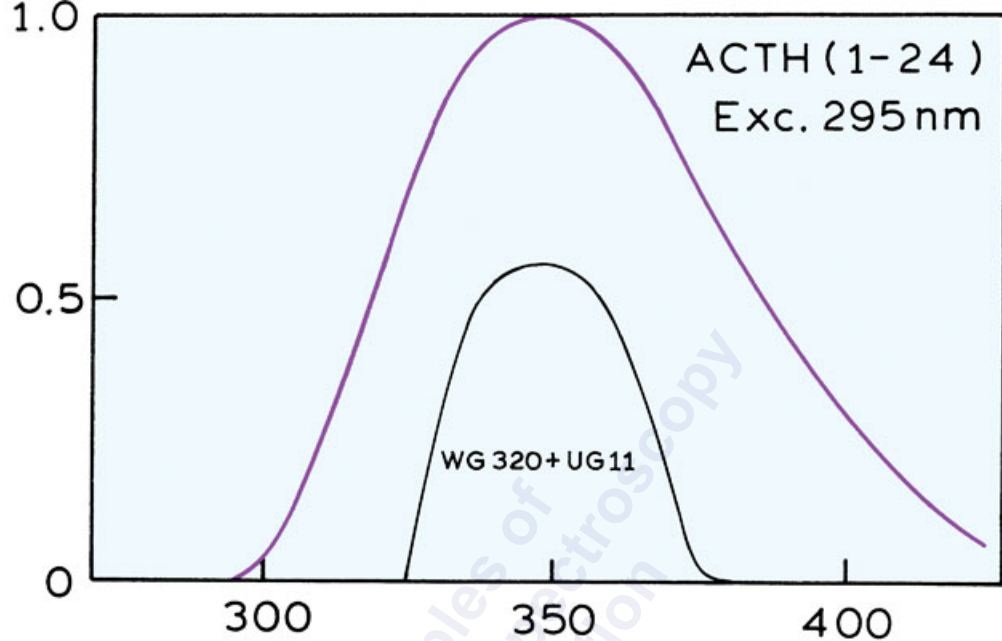
TRANSMISSION (%)

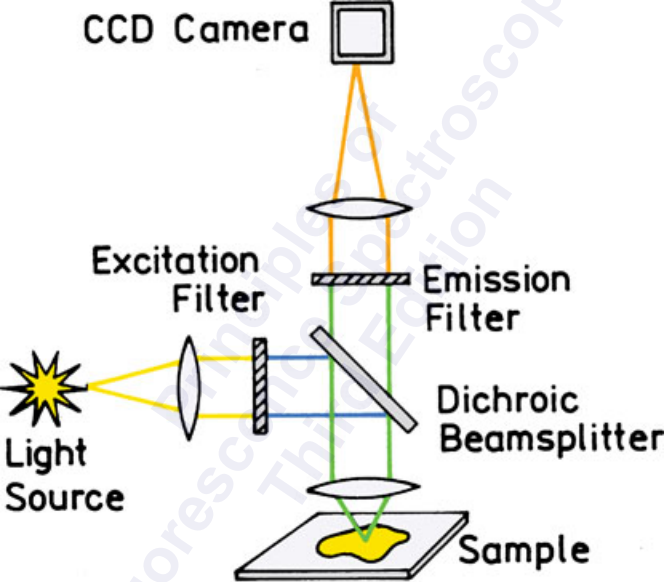




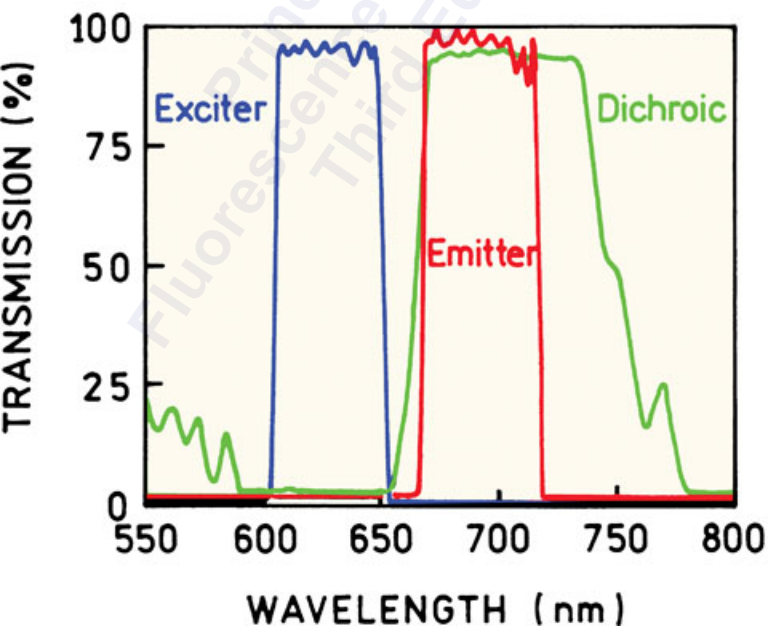
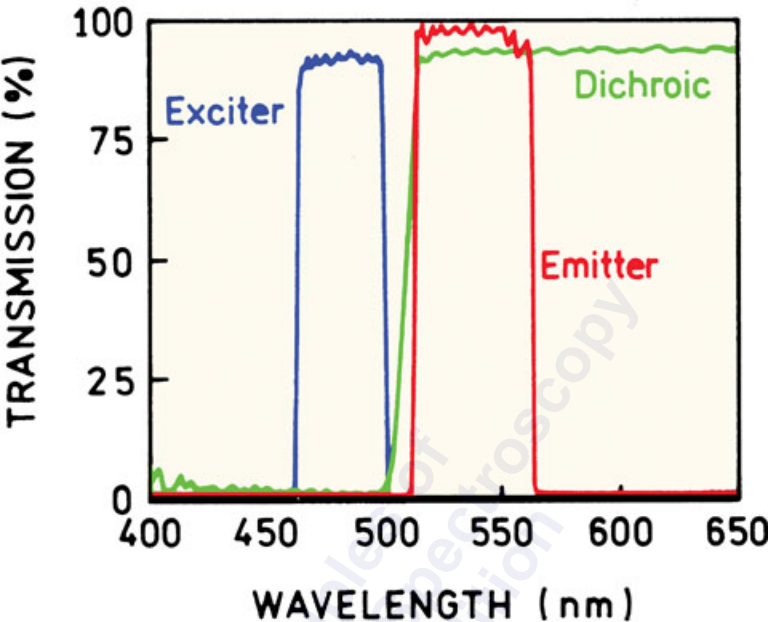


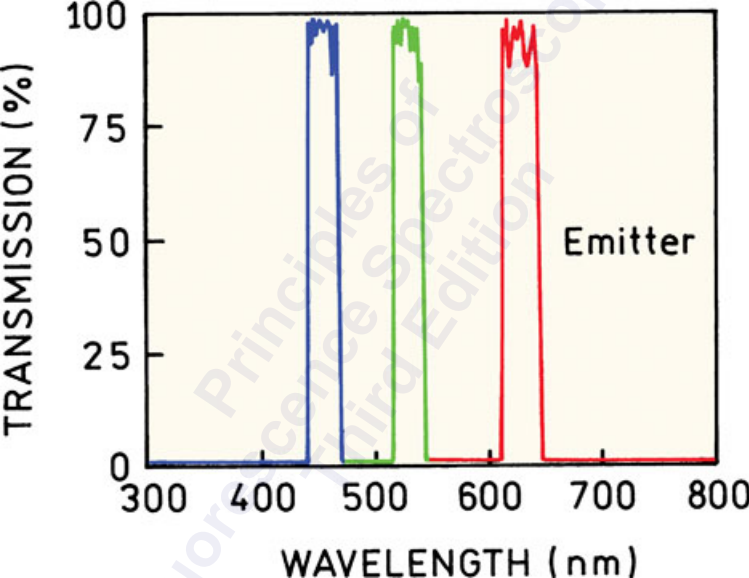
FLUORESCENCE INTENSITY

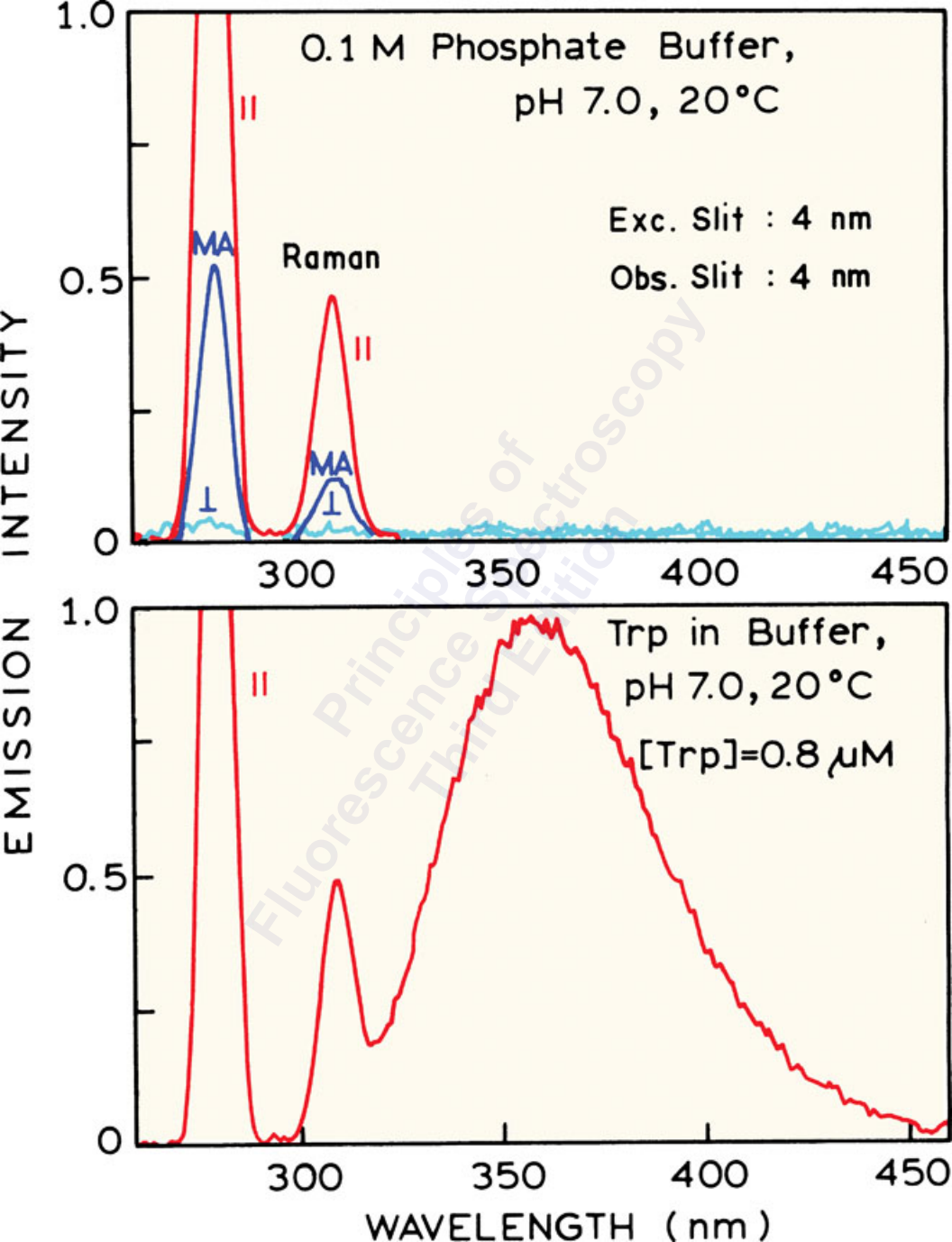








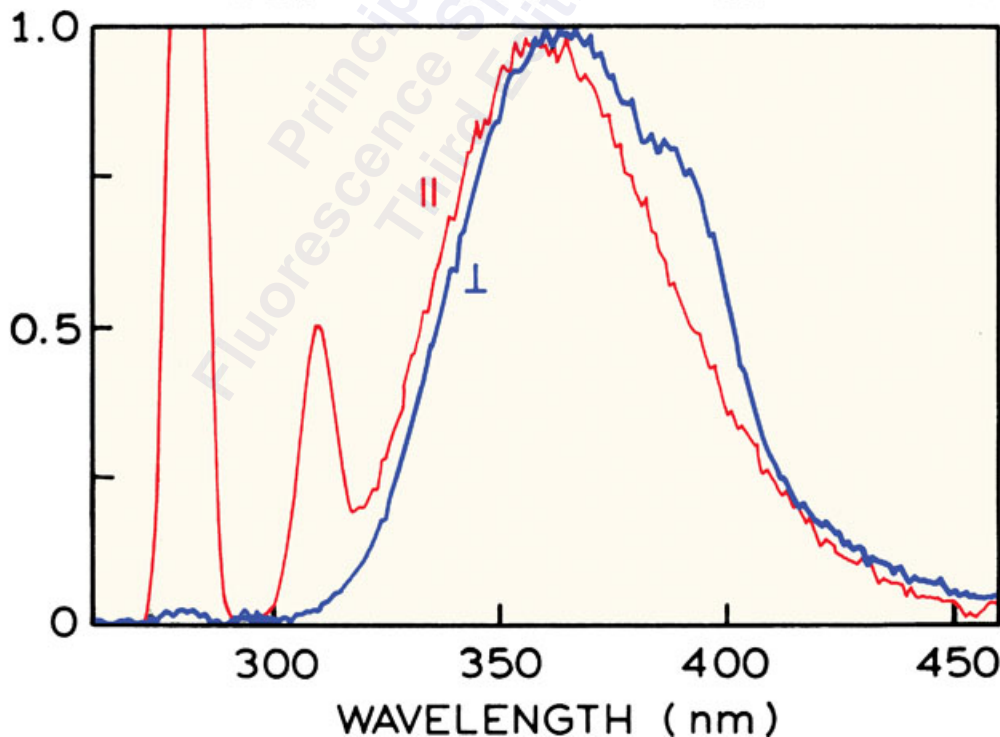
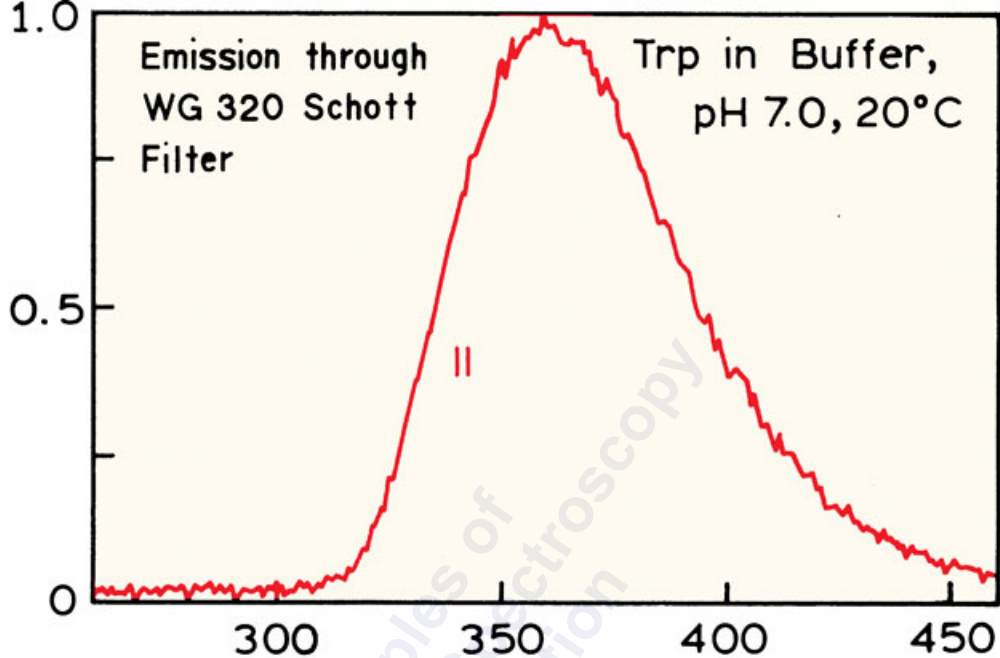


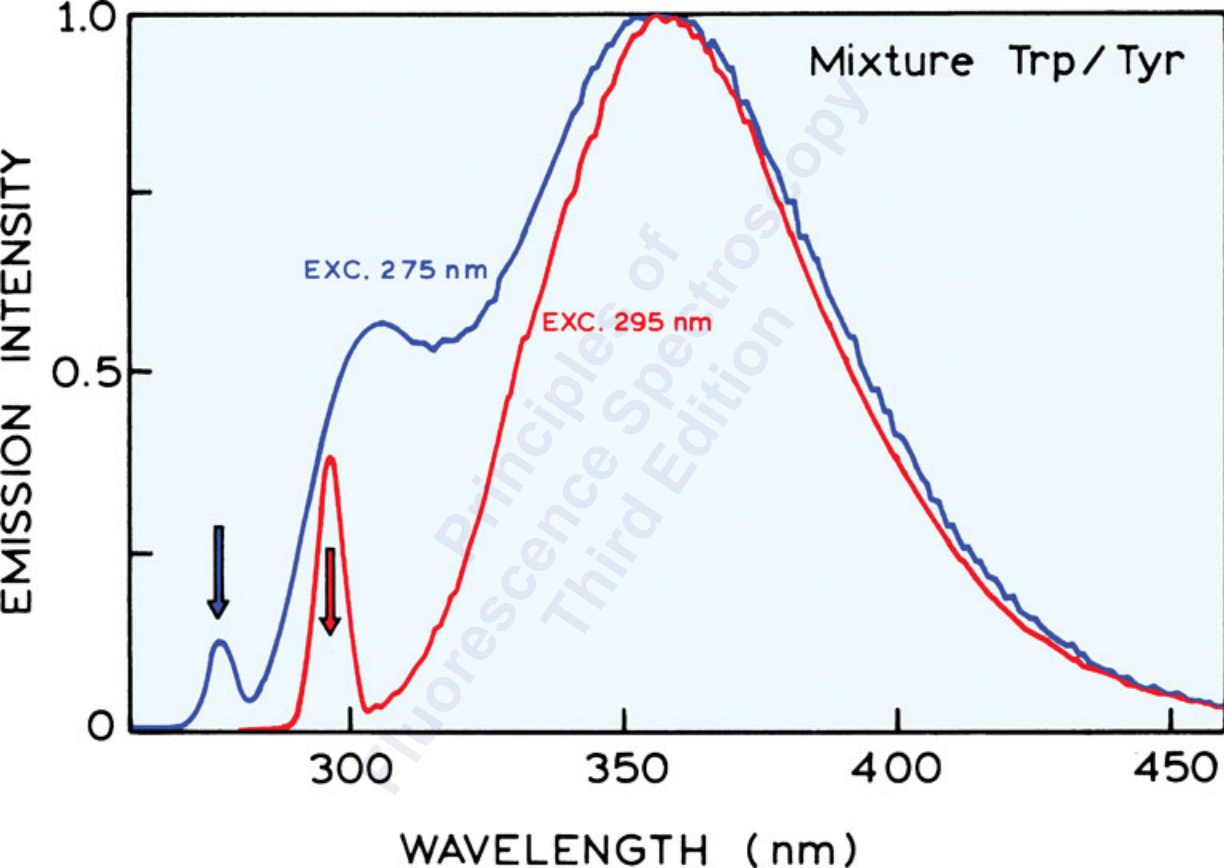


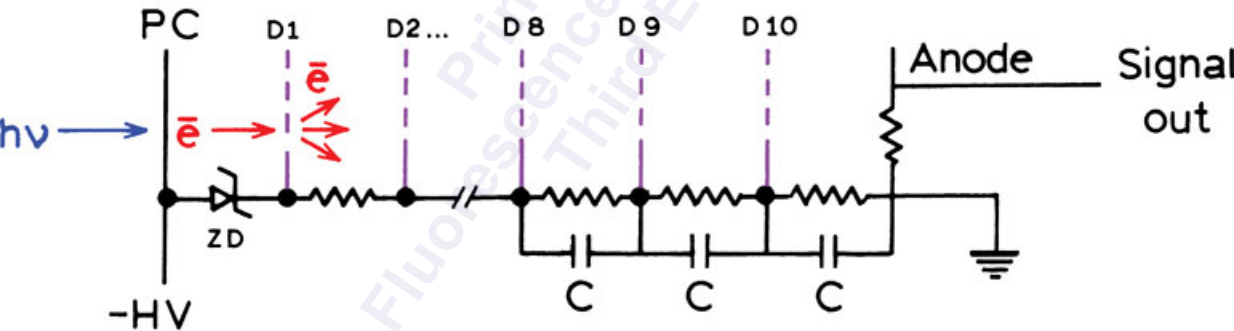
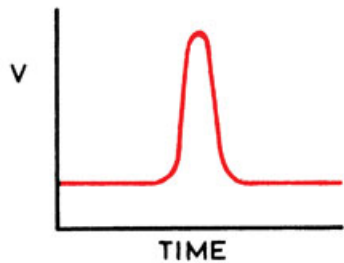
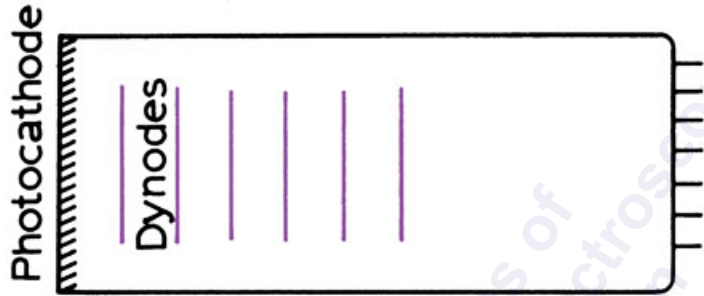
Emission through  
WG 320 Schott  
Filter

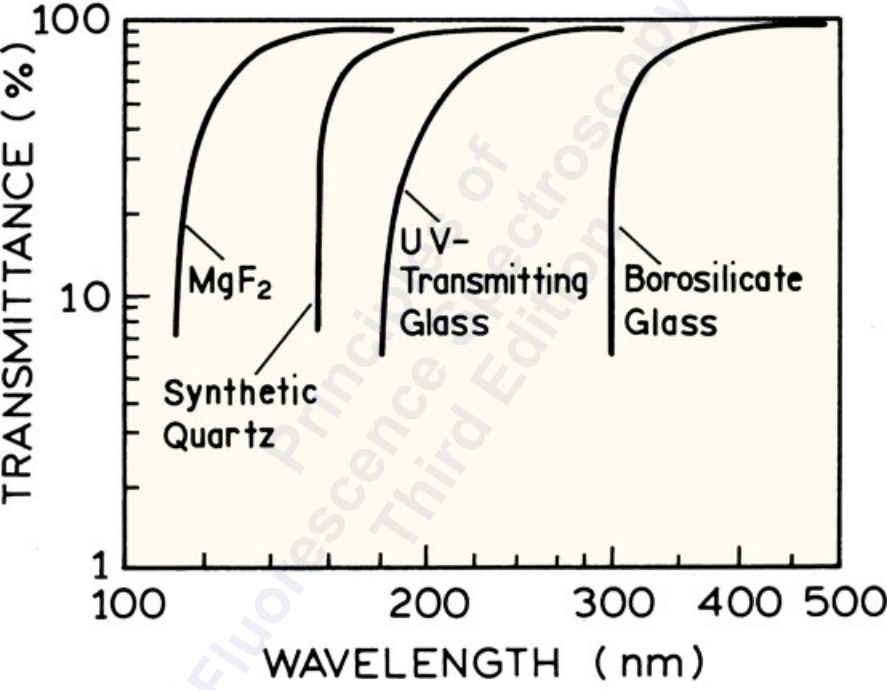
Trp in Buffer,  
pH 7.0, 20°C

EMISSION INTENSITY









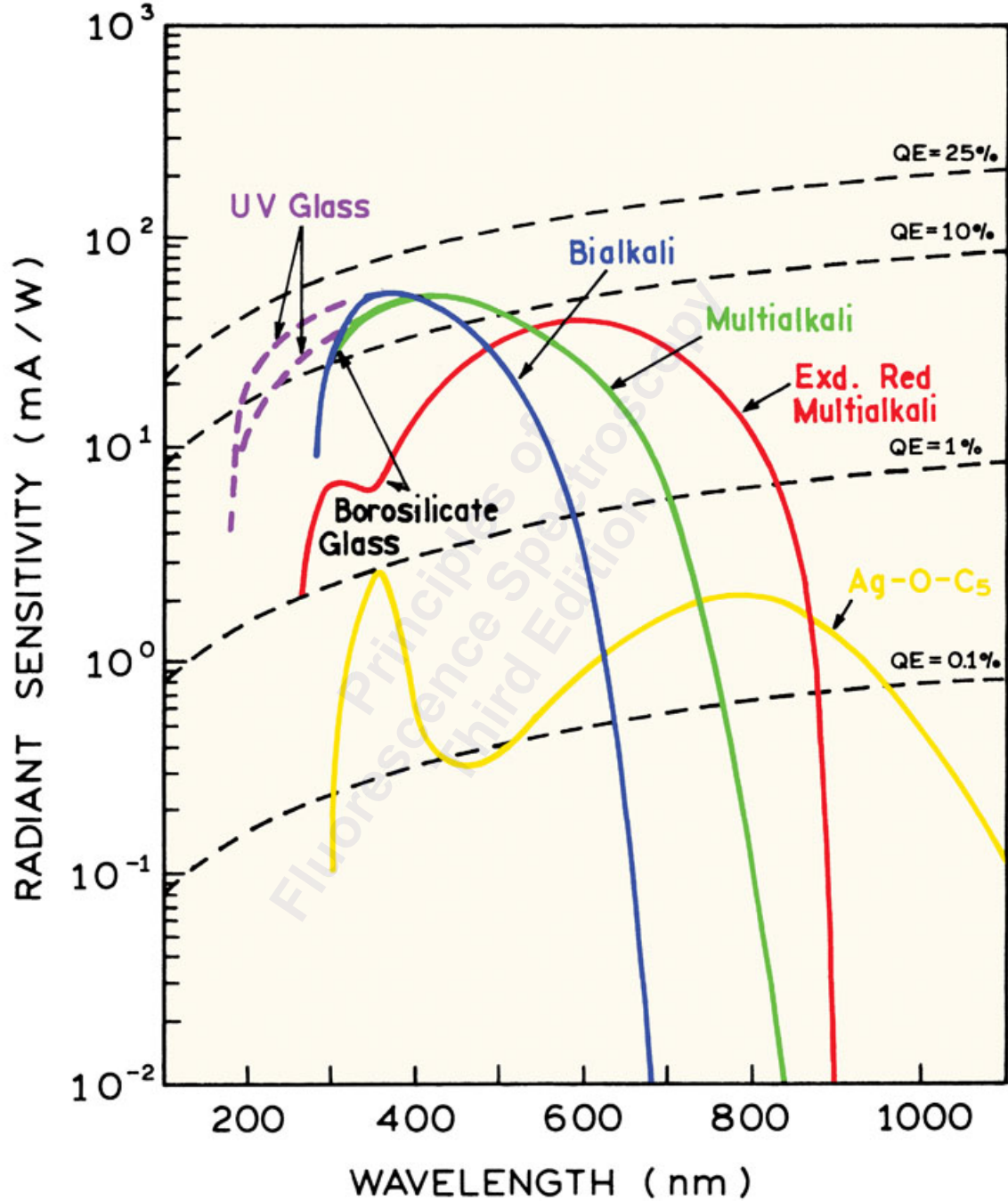




Photo -  
Sensitive  
Area

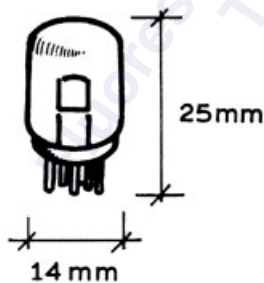


Side - On

Photo -  
Sensitive  
Area



Head - On



Sub - Miniature



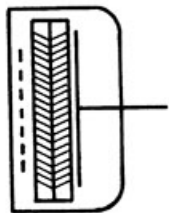
TO - 8



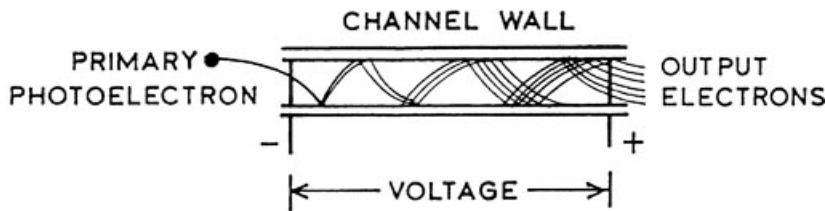
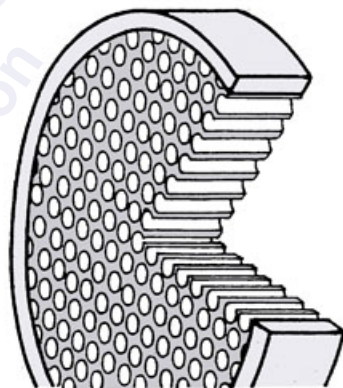
Circular cage

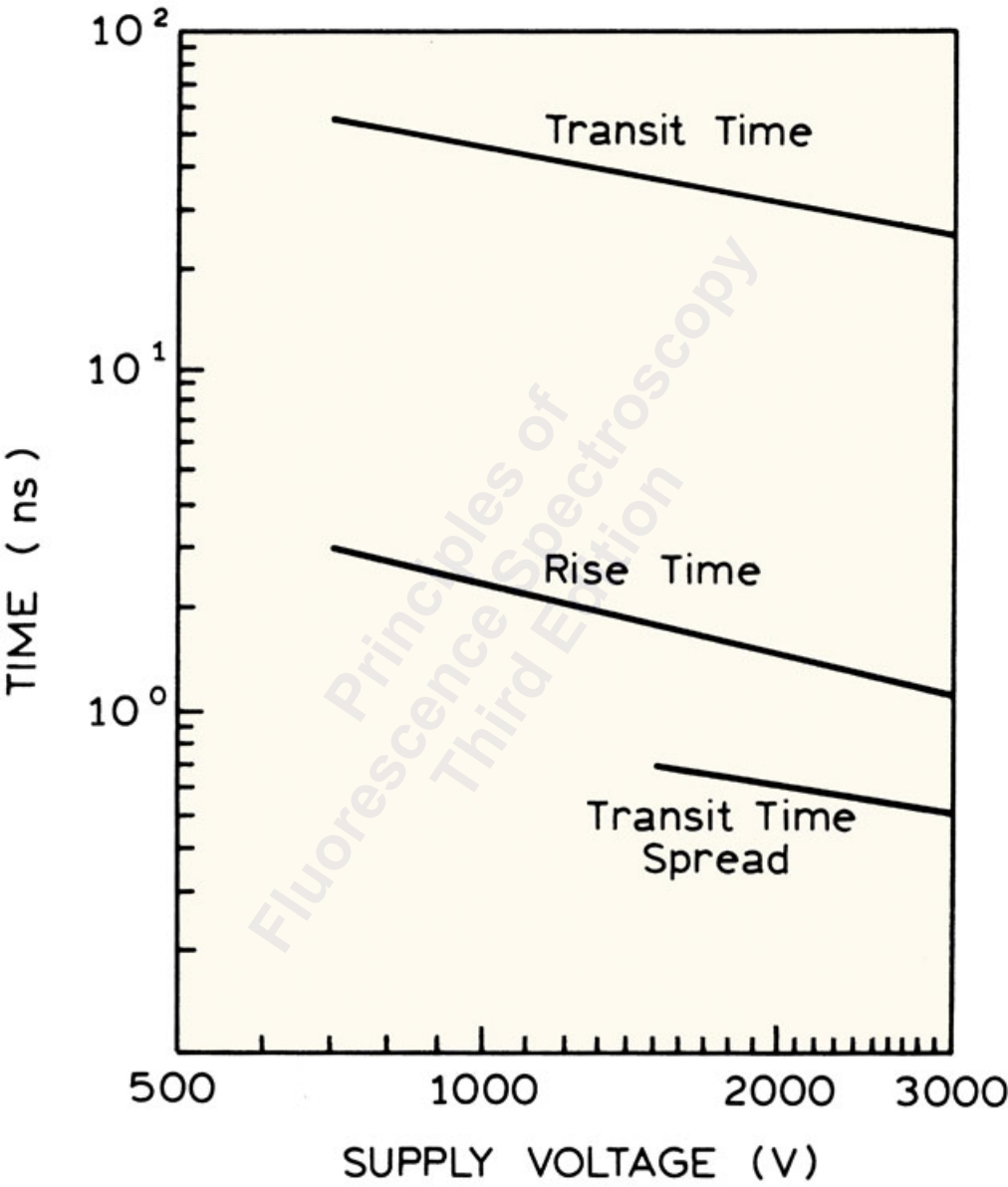


Linear Focused

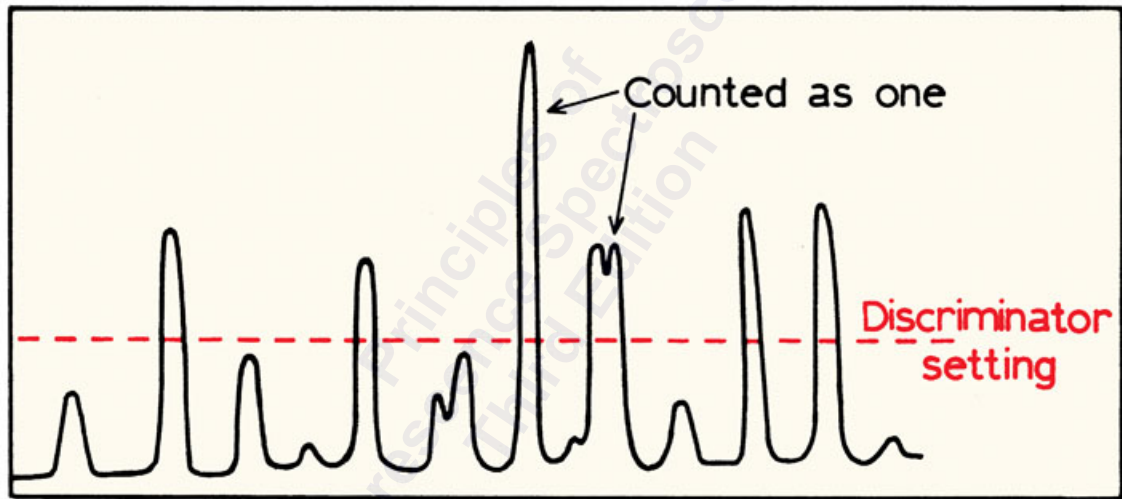


Microchannel plate

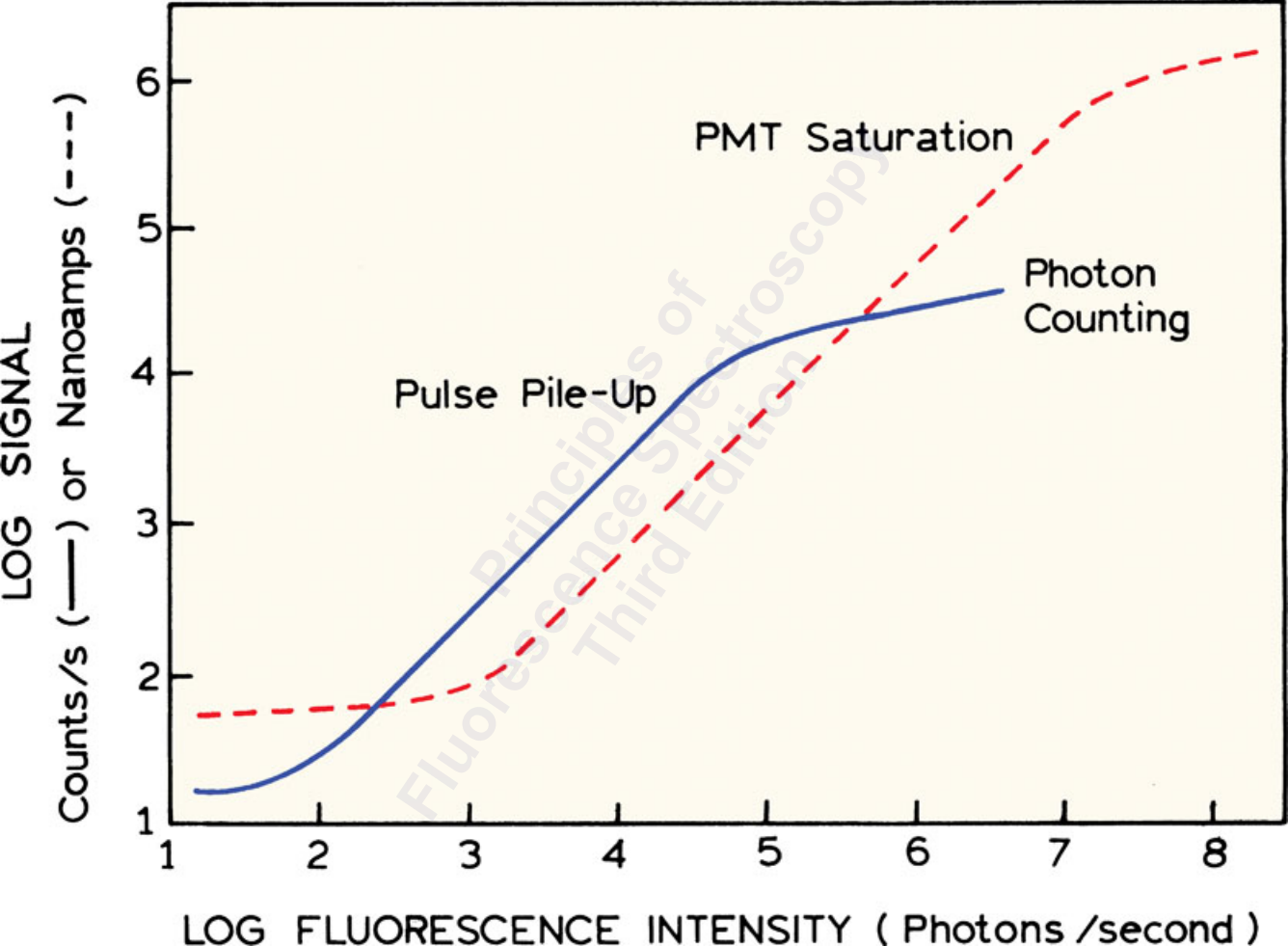


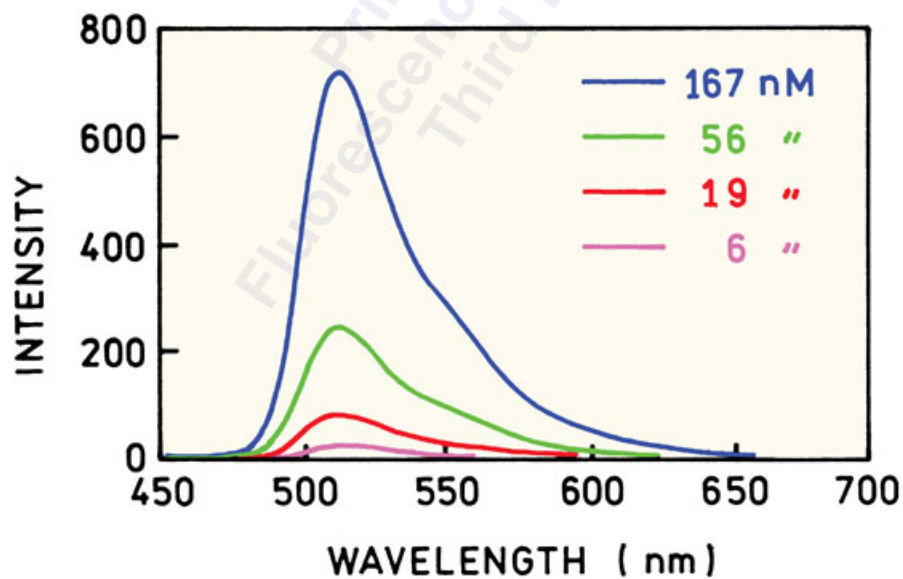


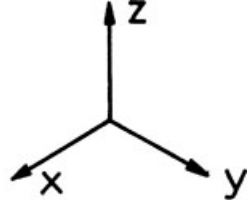
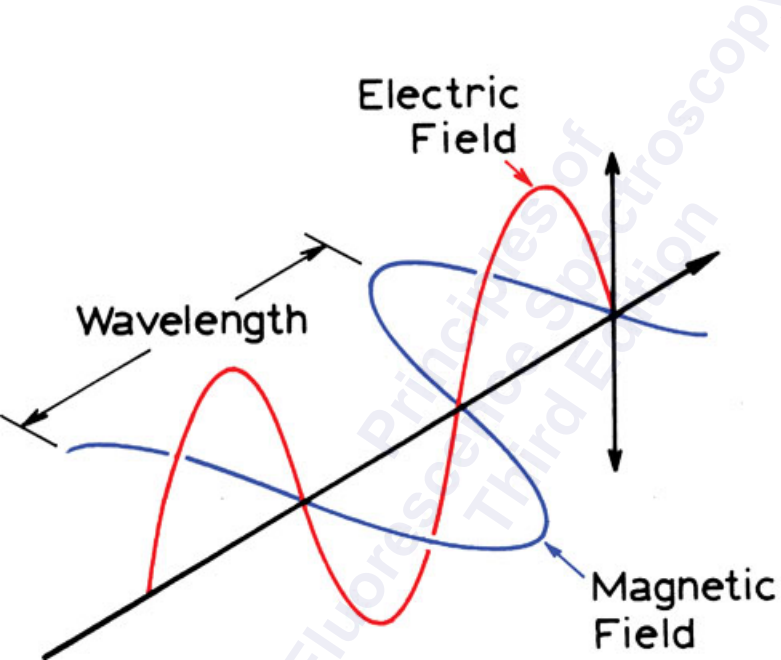
PMT ANODE OUTPUT (nA)



TIME (ns)







Incident  
Ray

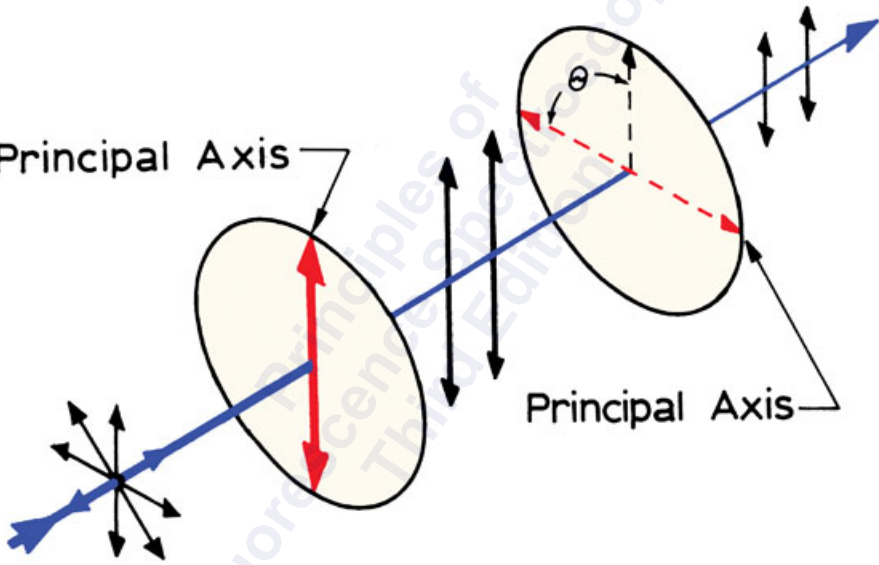
Optical  
Surface

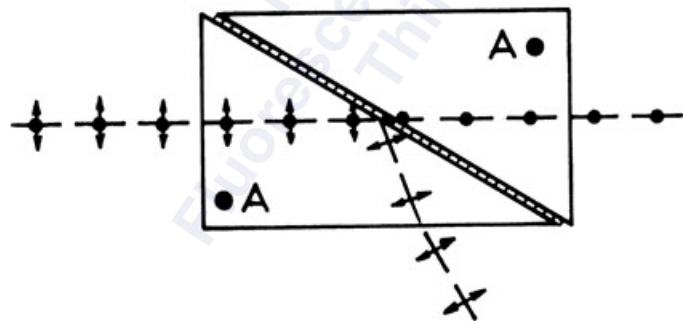
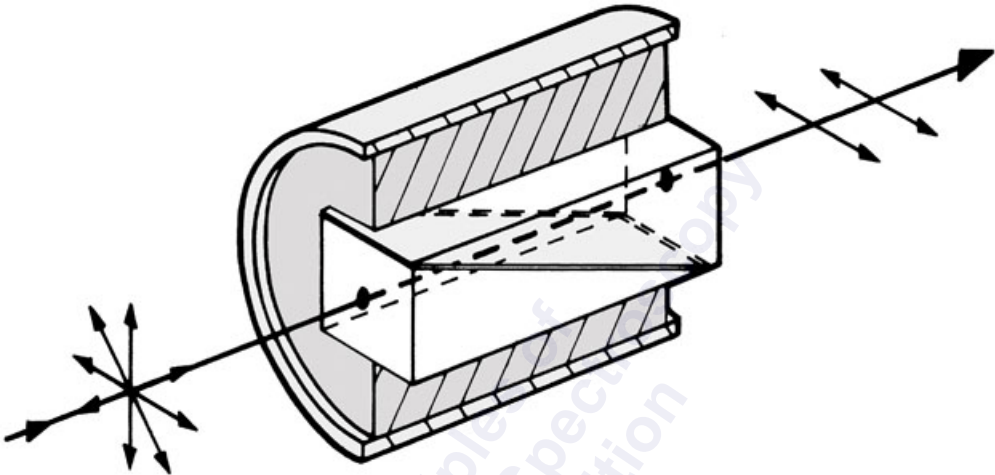




Principal Axis

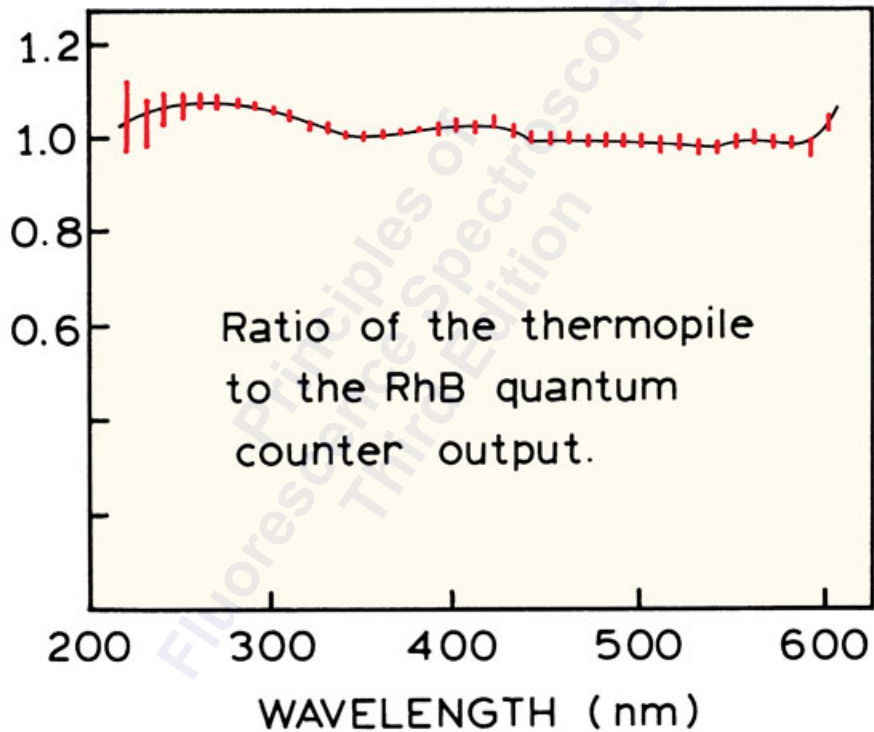
Principal Axis

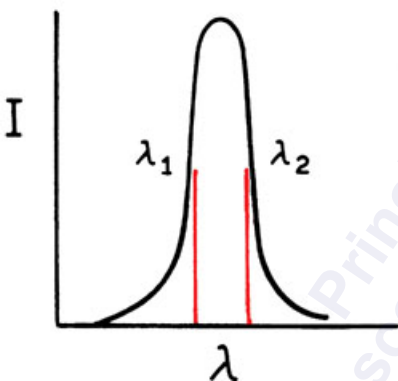




A = Crystal Optical Axis

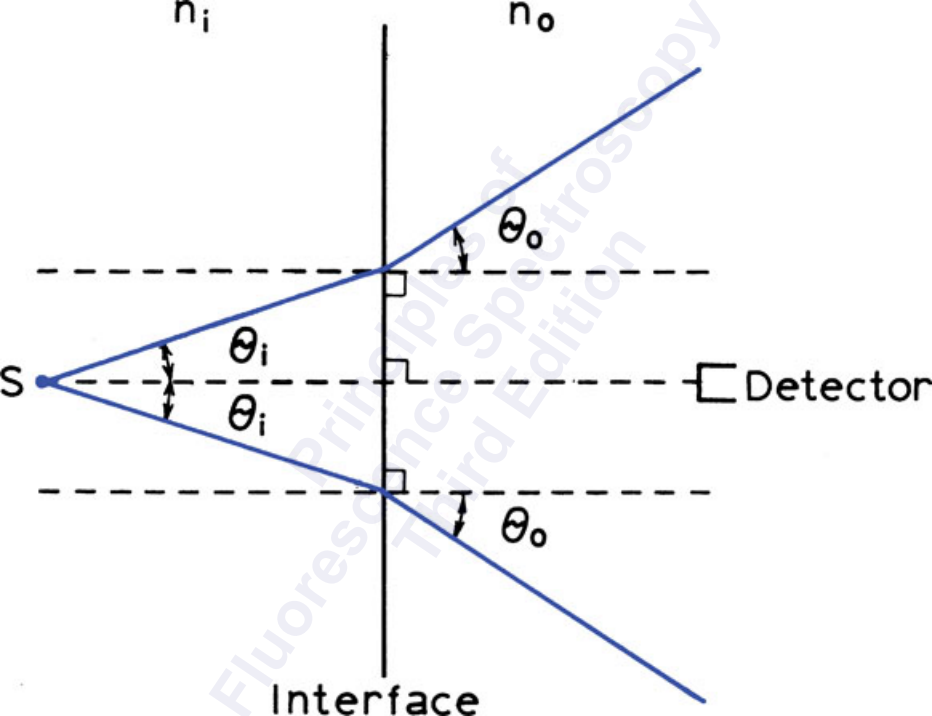
THERMOPILE/QUANTUM COUNTER

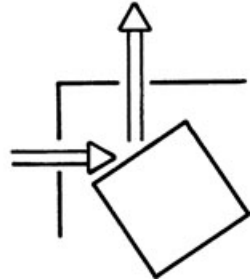
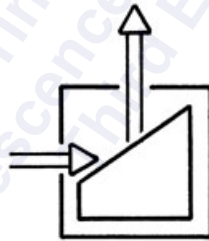
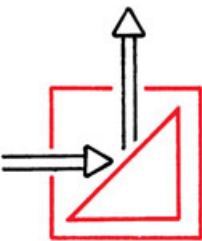
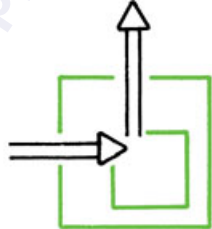
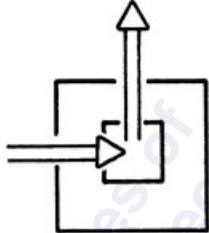
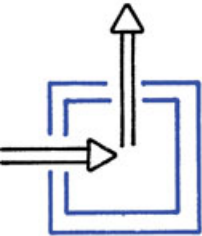


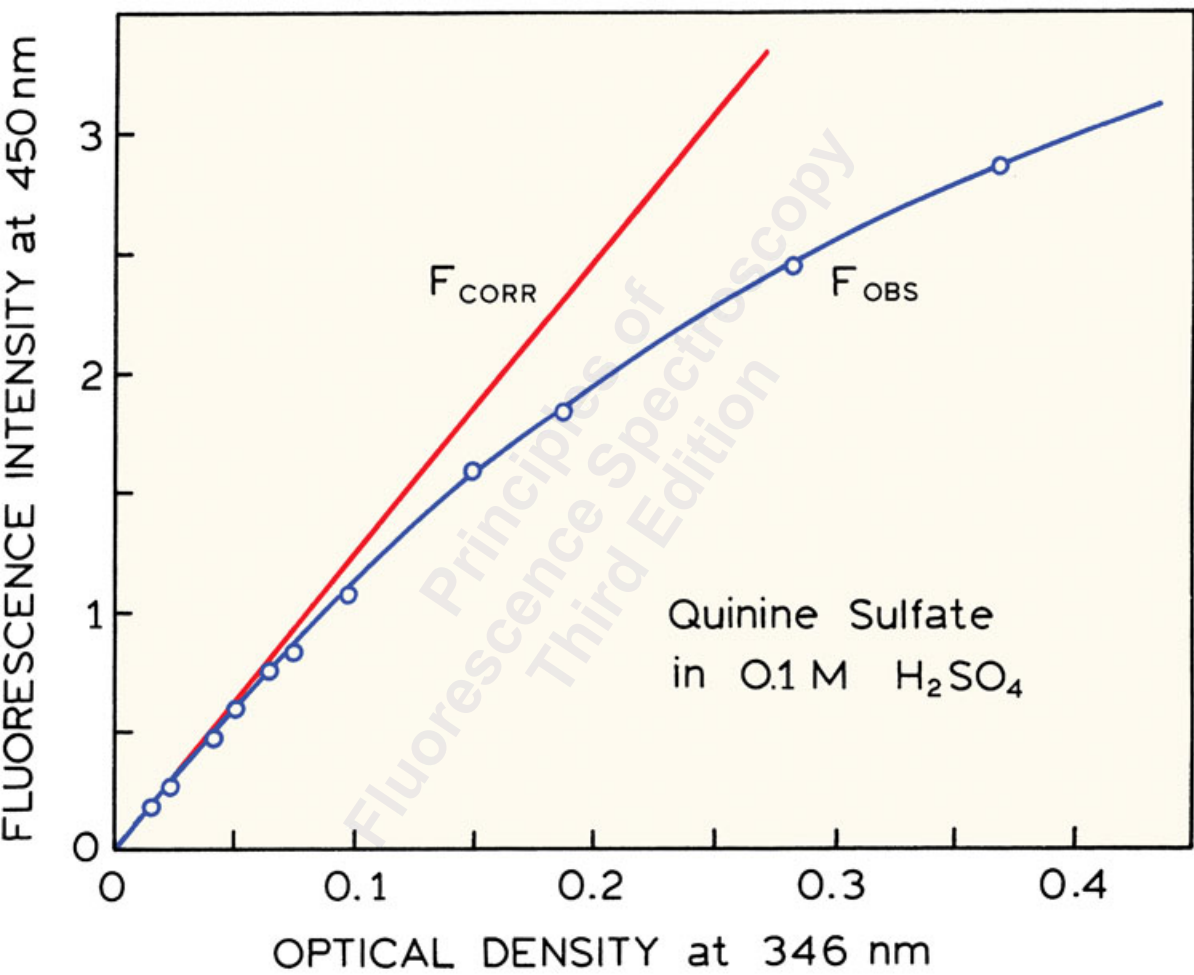


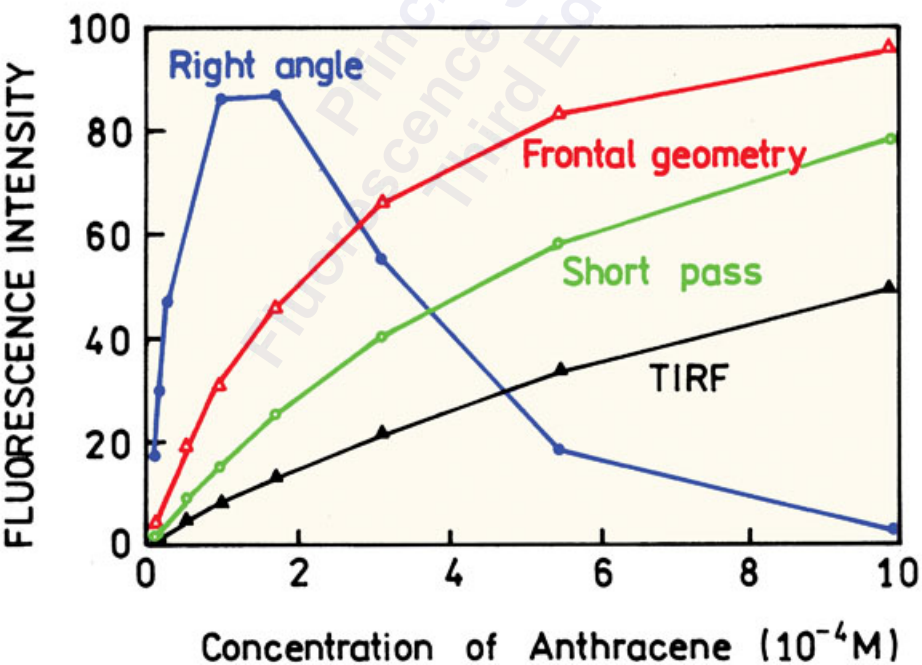
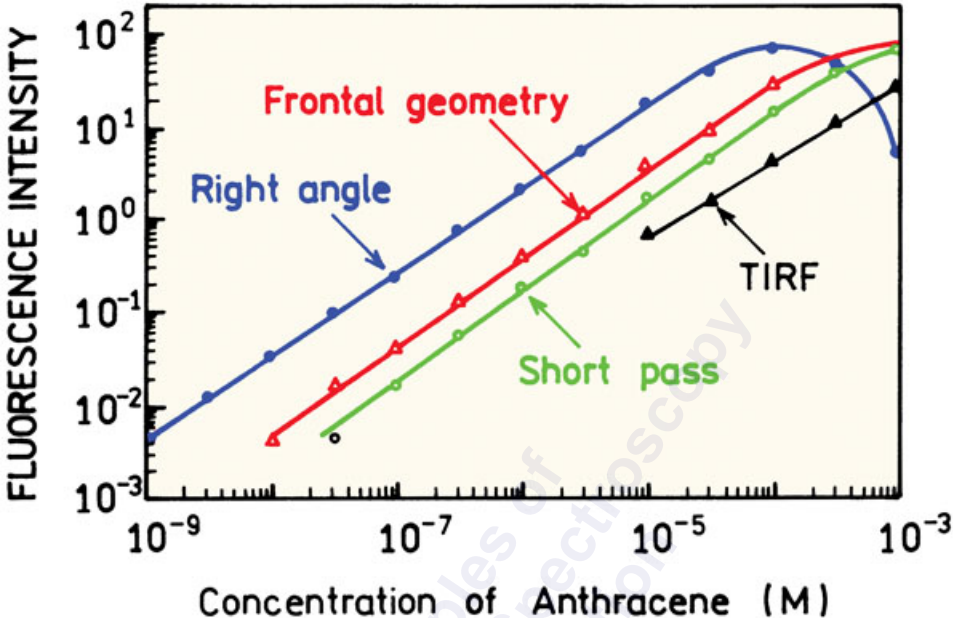
$$\Delta \bar{\nu} = \bar{\nu}_2 - \bar{\nu}_1 = \frac{1}{\lambda_2} - \frac{1}{\lambda_1}$$

$$= \frac{\lambda_1 - \lambda_2}{\lambda_1 \lambda_2} \approx \frac{\Delta \lambda}{\lambda^2}$$











FLUORESCENCE INTENSITY

Anthracene

6

$10^{-6}M$

$10^{-5}M$

$10^{-4}M$

$10^{-3}M$

2

0

360

380

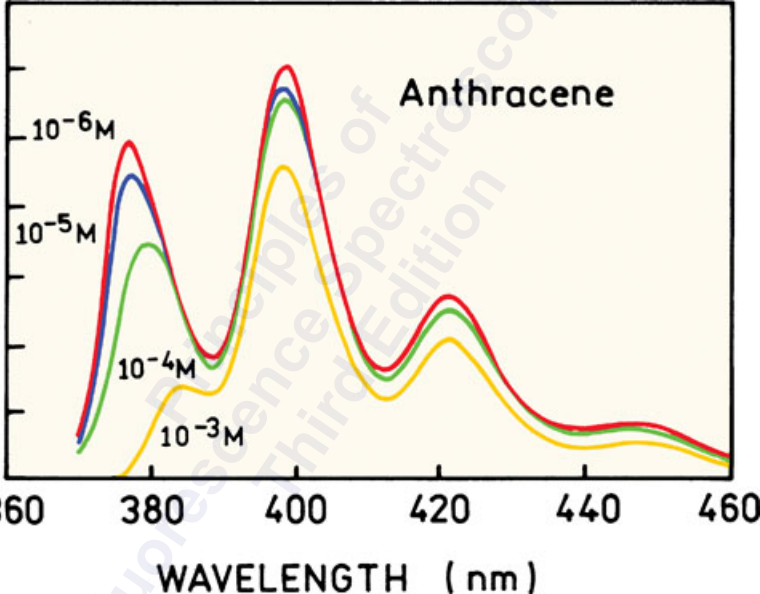
400

420

440

460

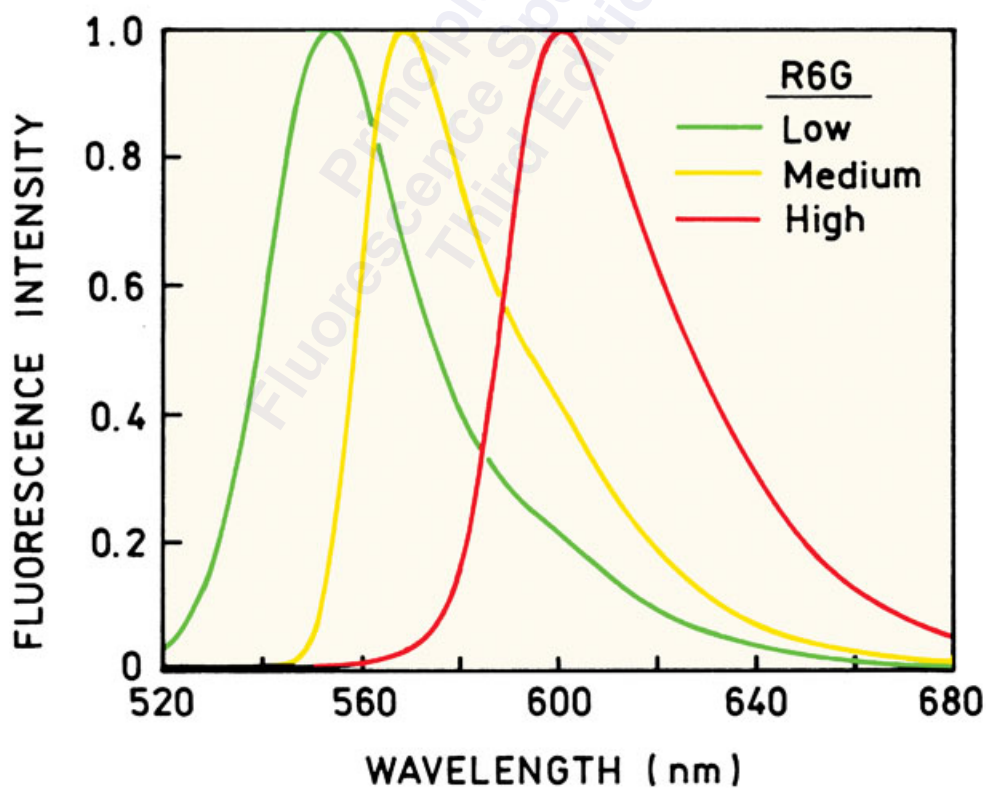
WAVELENGTH (nm)



Low

Medium

High



Fluorophore concentration too high

Wrong



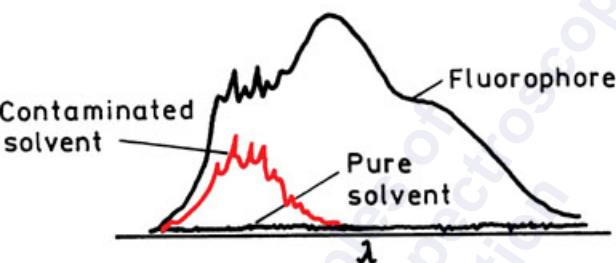
Obs. slit

Right

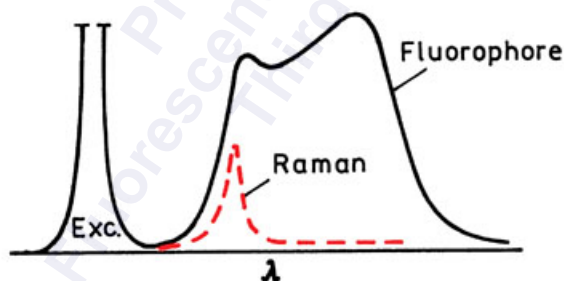


Obs. slit

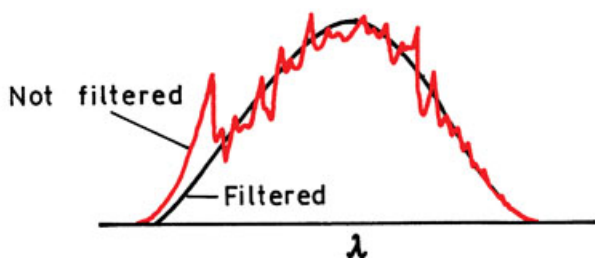
Contaminated solvent and/or cuvette

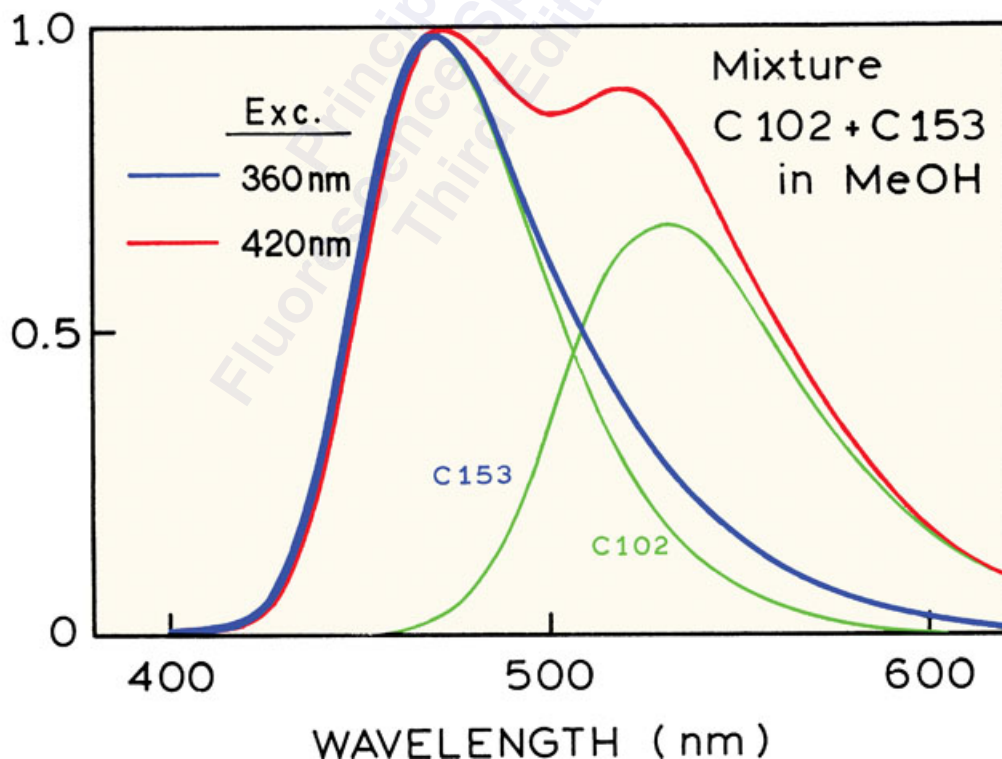
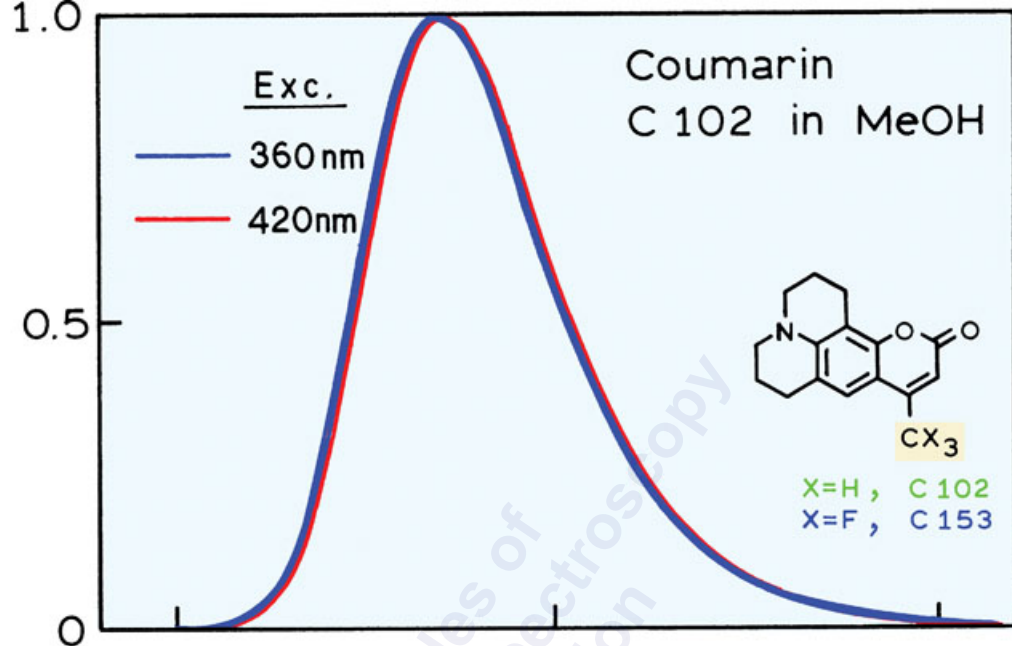


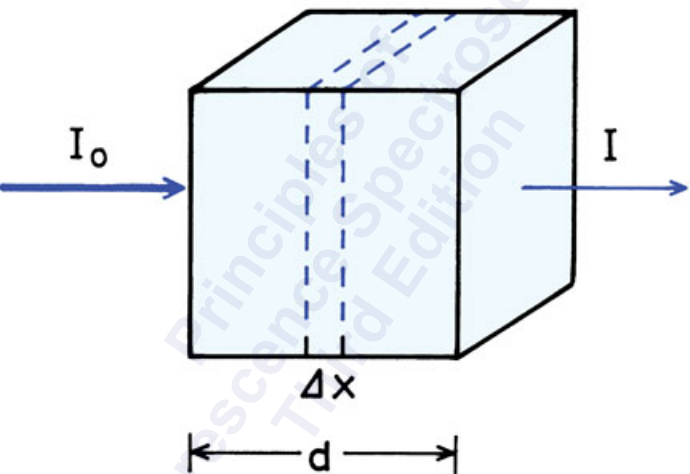
Scattered light

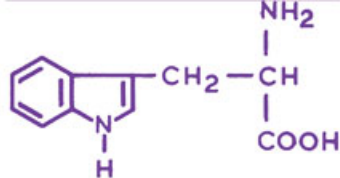


Particles in solution

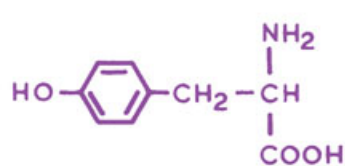




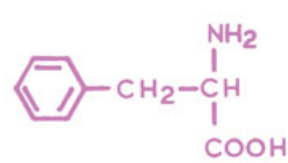




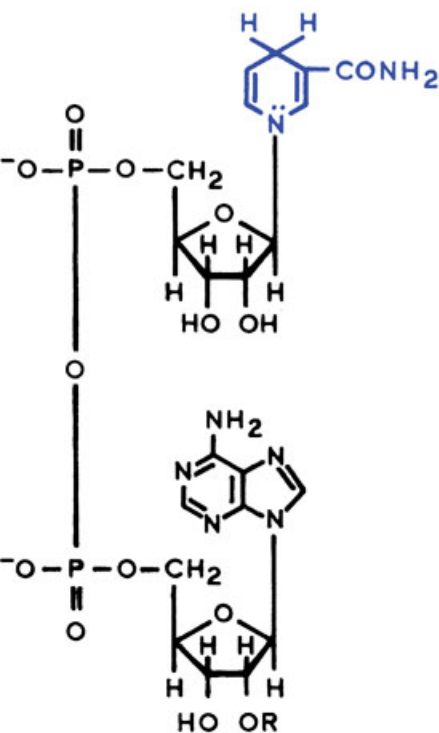
Tryptophan



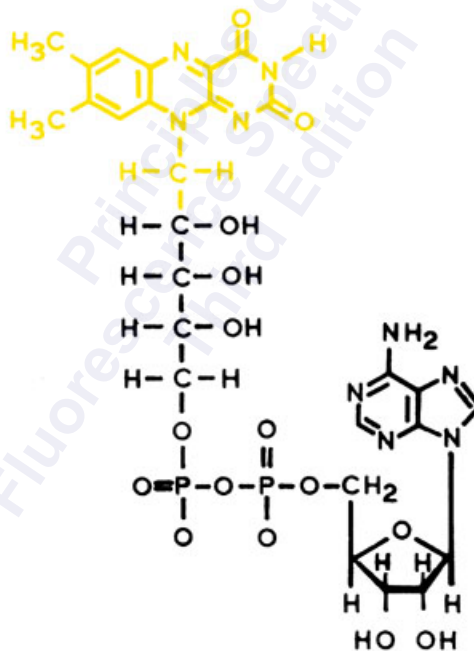
Tyrosine



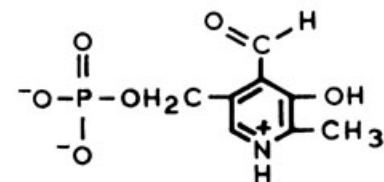
Phenylalanine



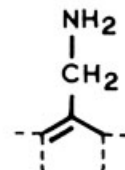
NADH



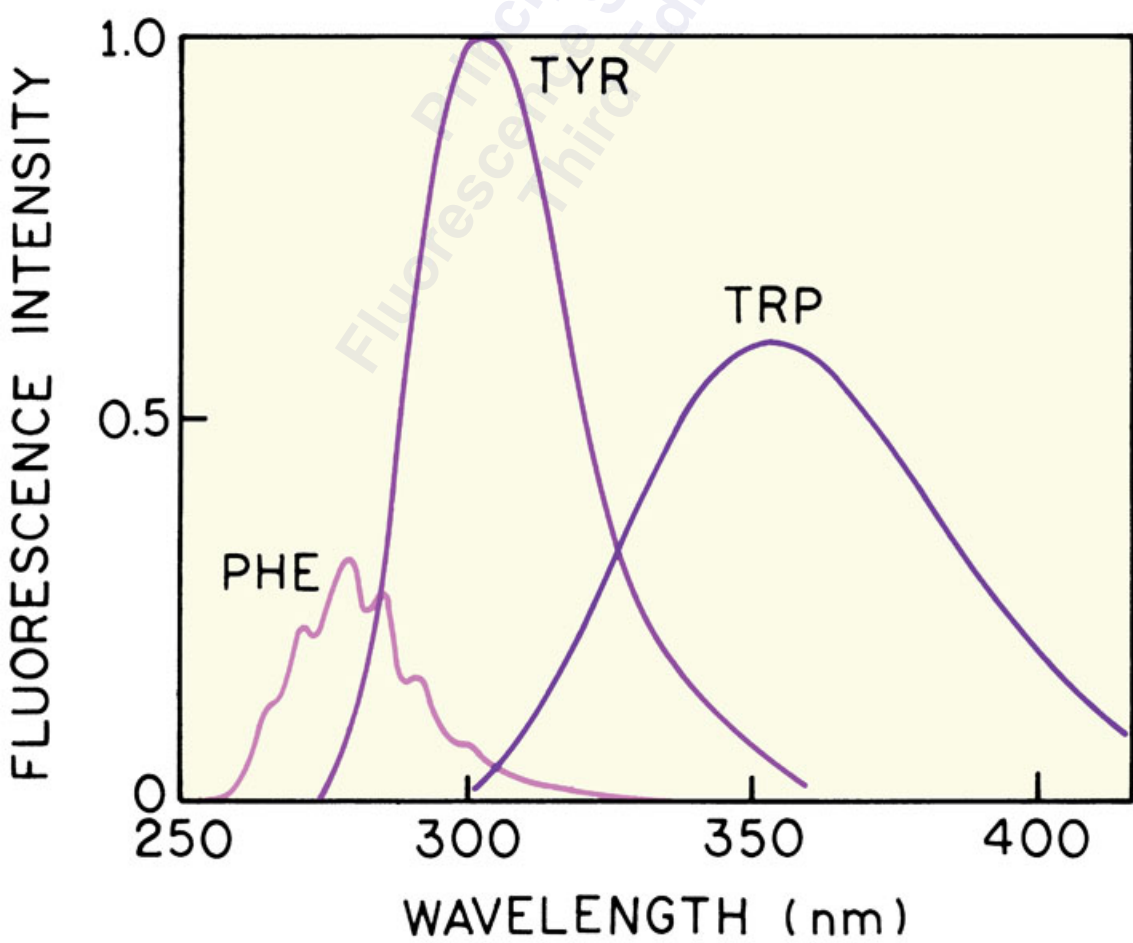
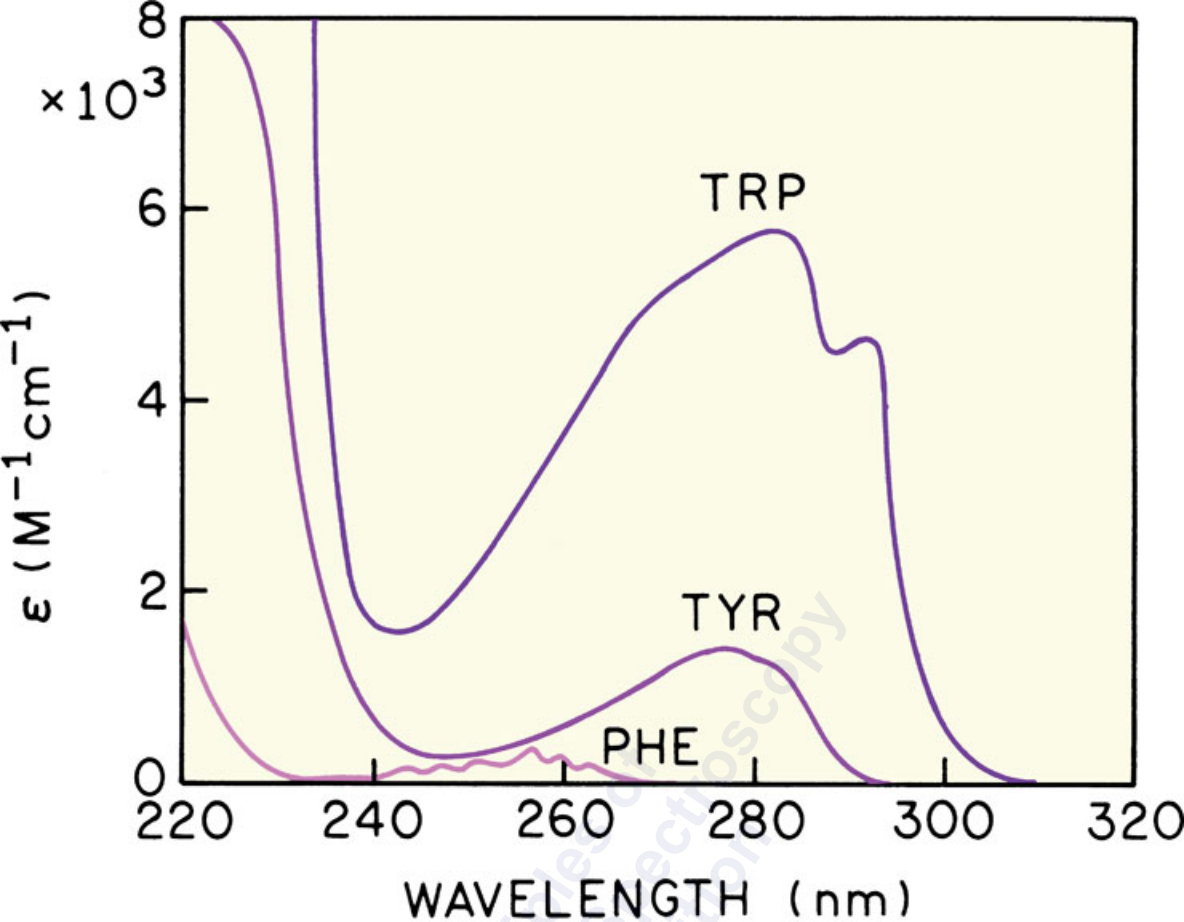
FAD



Pyridoxal  
phosphate



Pyridoxamine  
phosphate

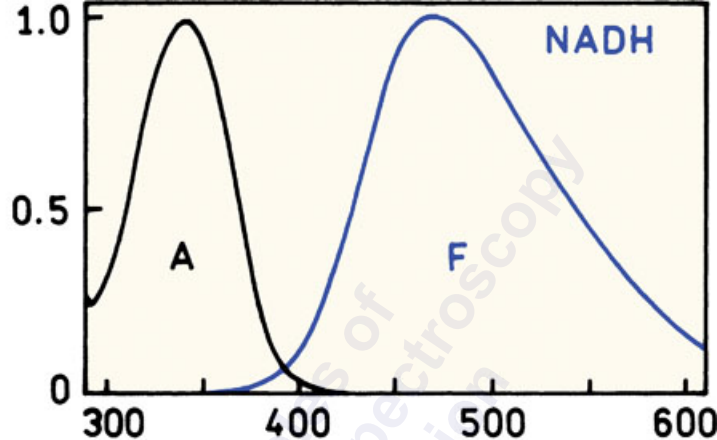


ABSORPTION or FLUORESCENCE

NADH

A

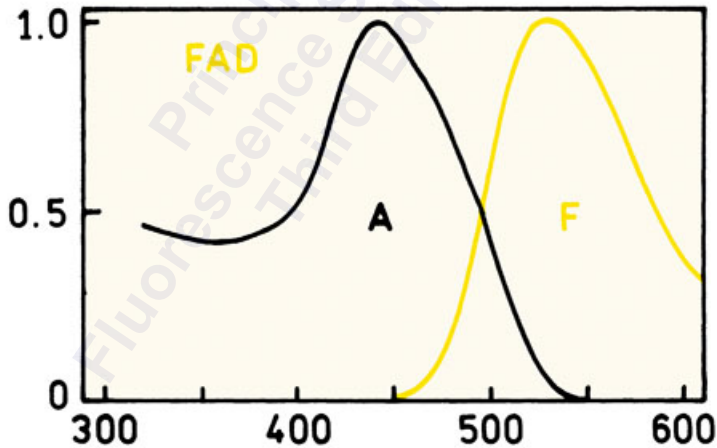
F



FAD

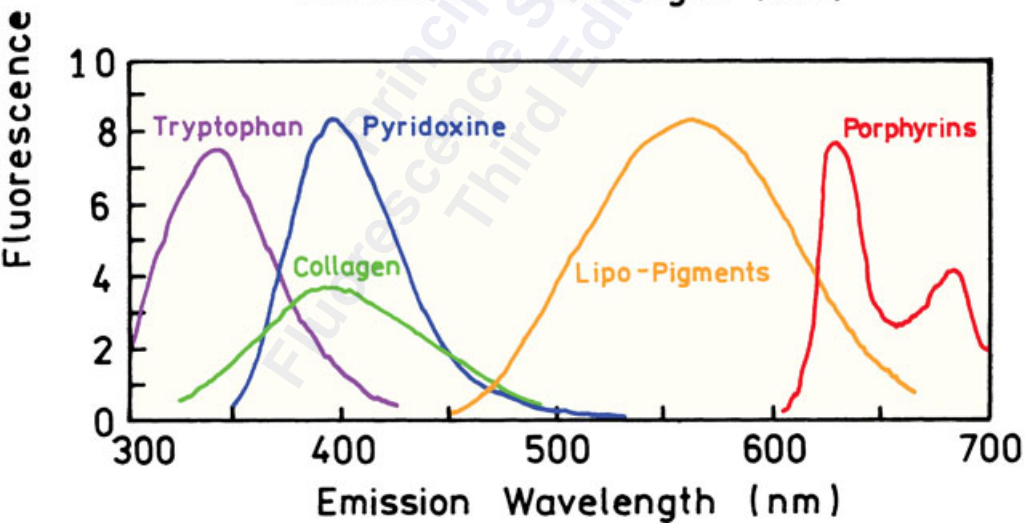
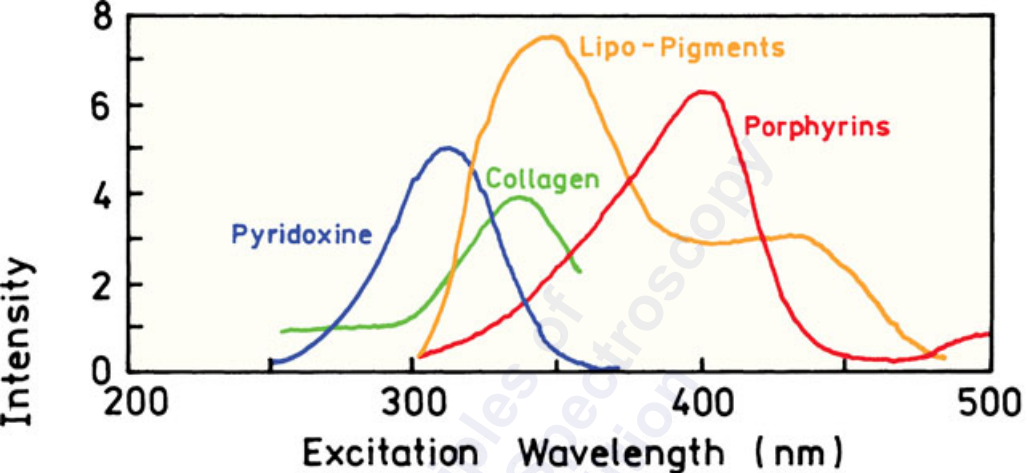
A

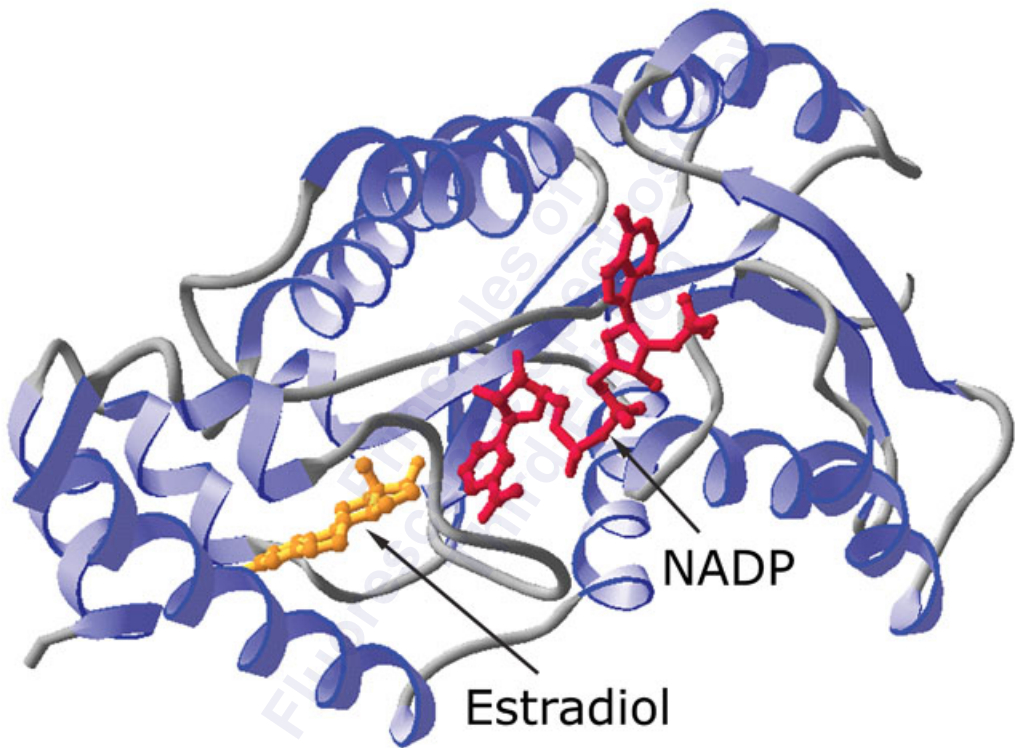
F

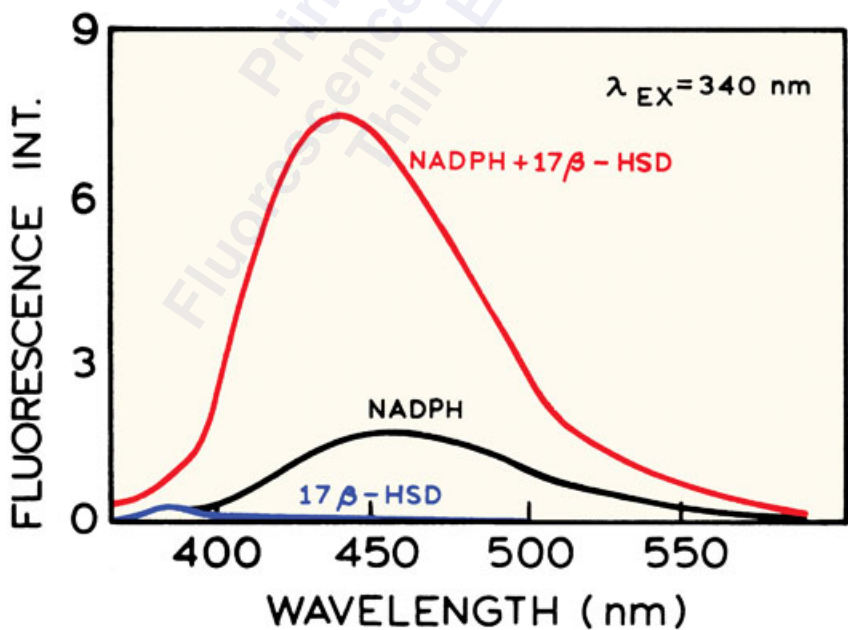
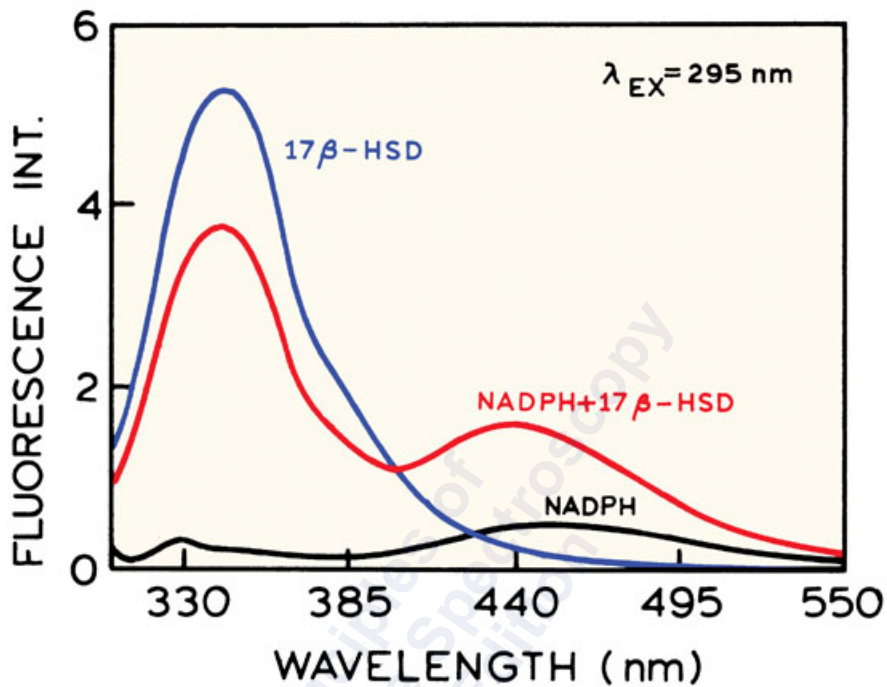


WAVELENGTH (nm)

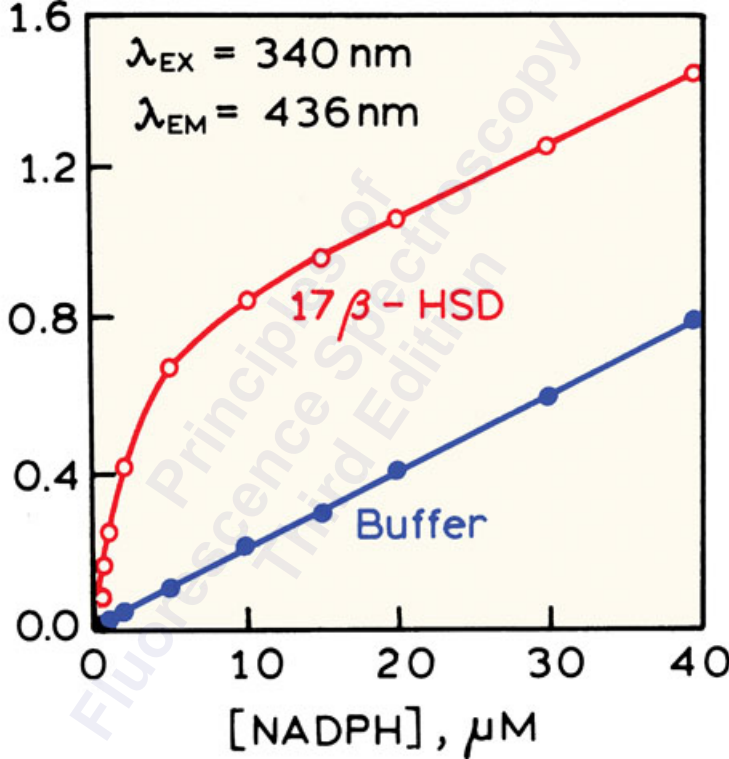


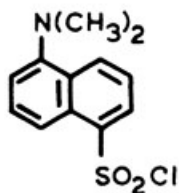




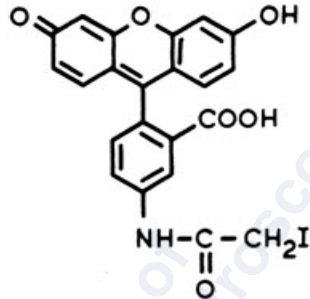


RELATIVE FLUORESCENCE

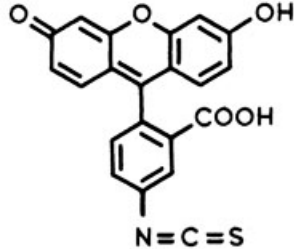




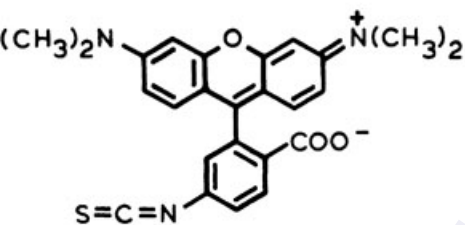
DNS-Cl



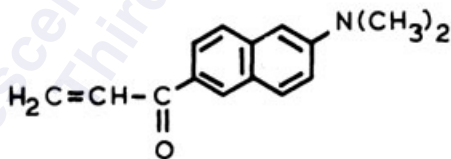
5-IAF



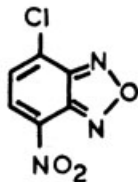
FITC



TRITC

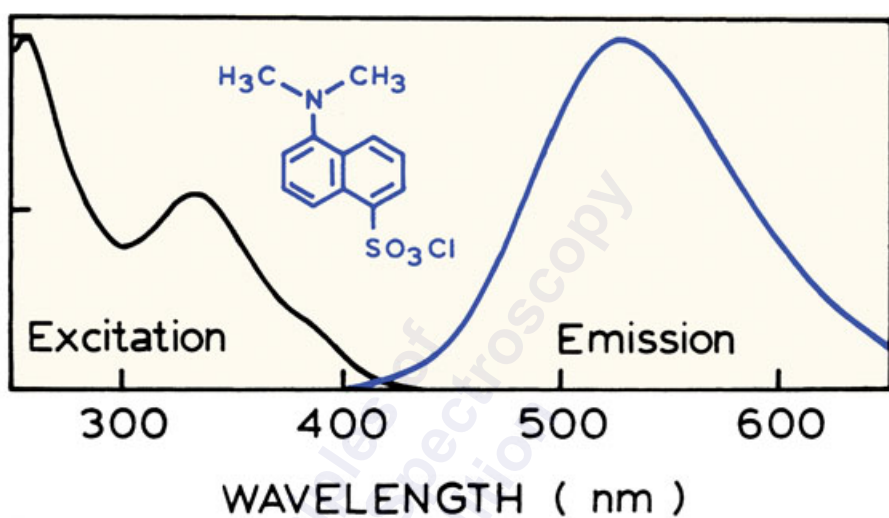
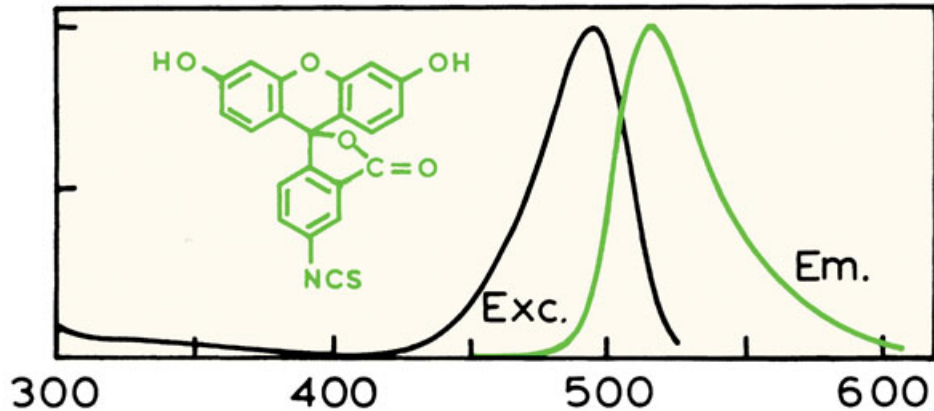


Acrylodan

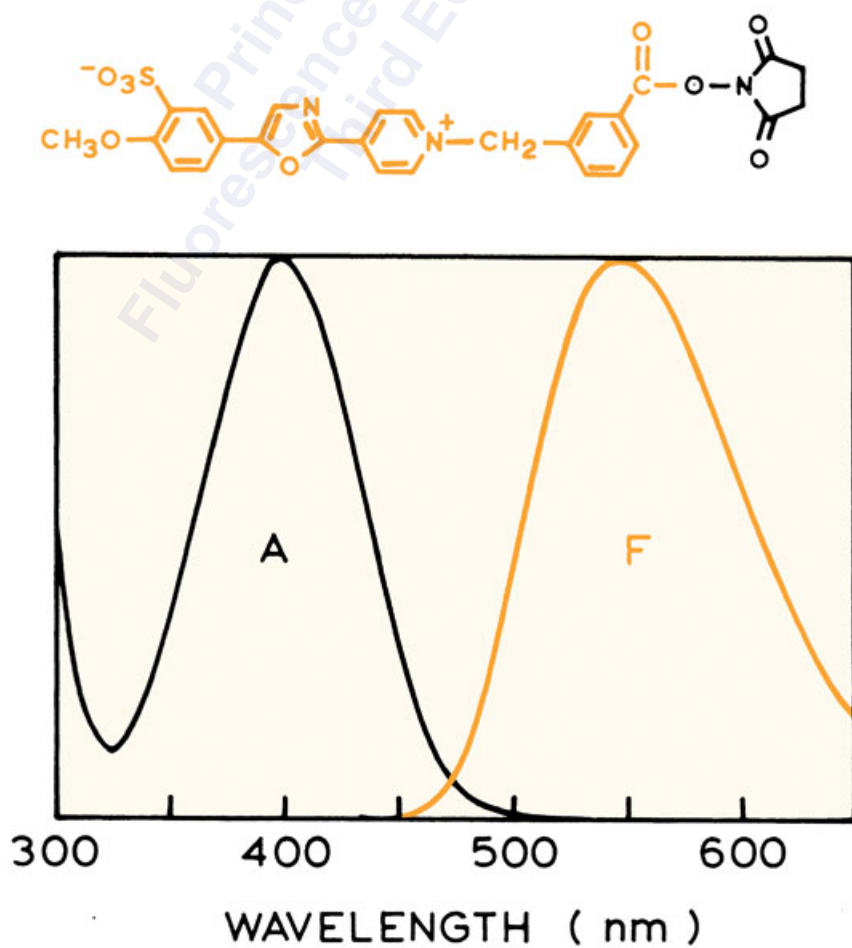


NBD-Cl

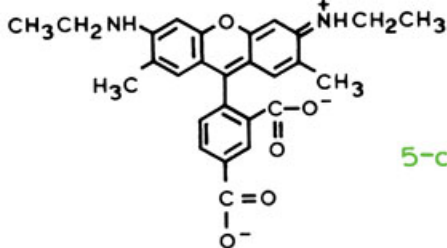
RELATIVE FLUORESCENCE



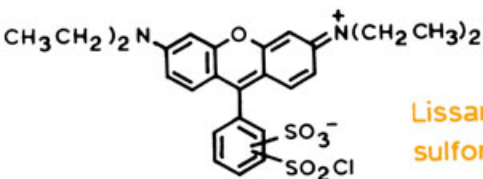
ABSORPTION OF  
FLUORESCENCE



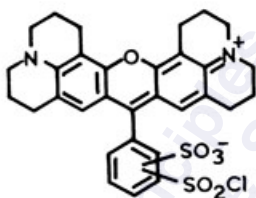




5-carboxyrhodamine 6G  
hydrochloride

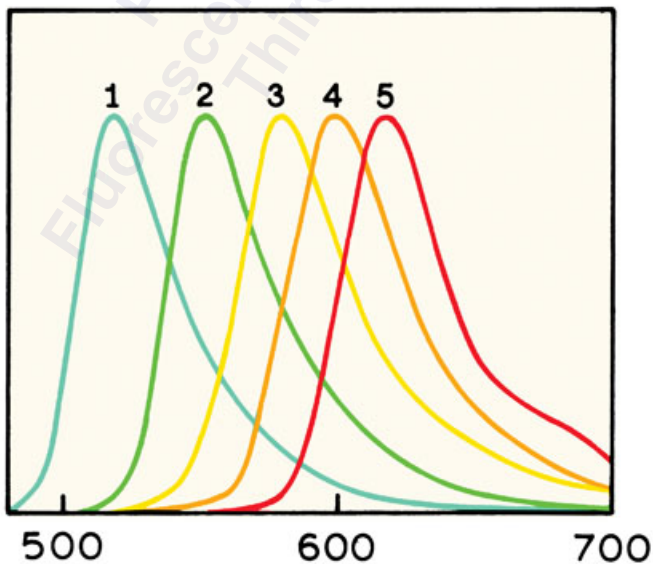


Lissamine rhodamine B  
sulfonyl chloride

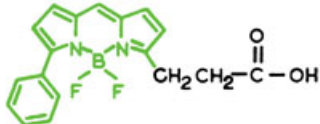


Texas Red  
sulfonyl chloride

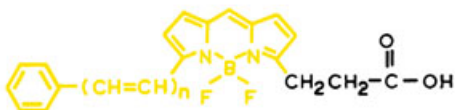
FLUORESCENCE EMISSION



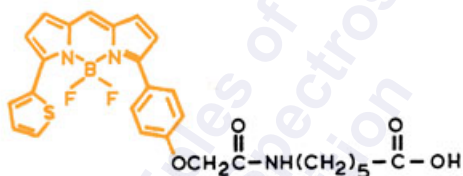




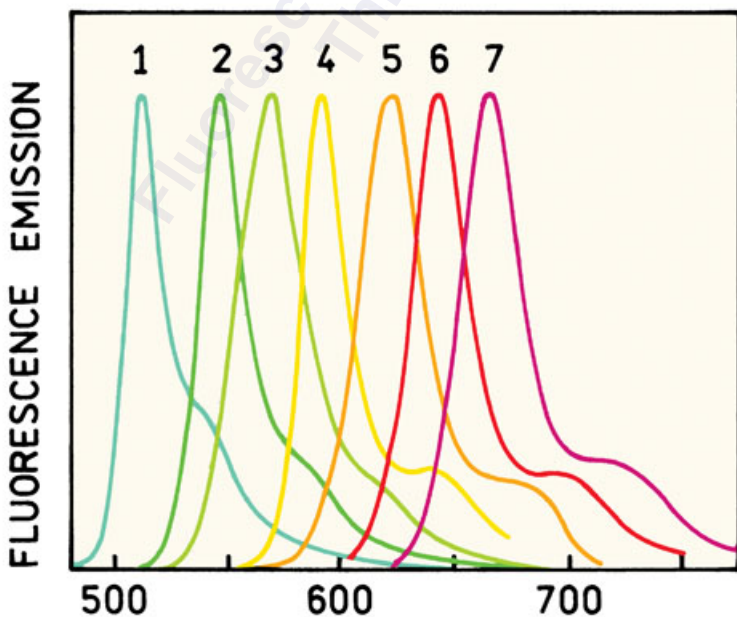
BODIPY-R6G

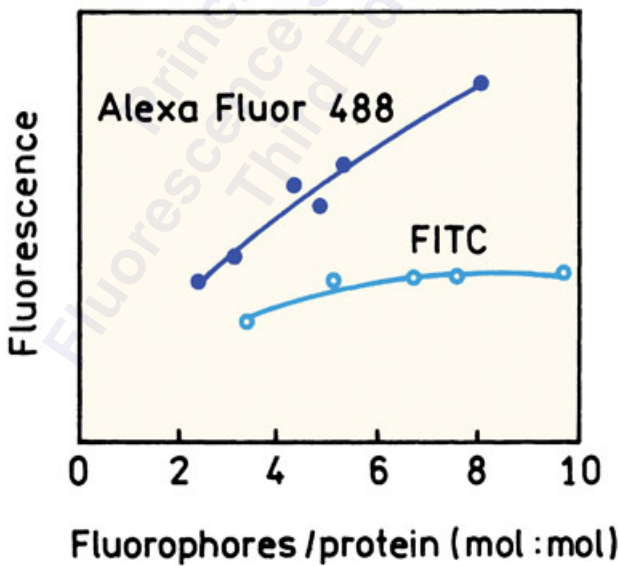
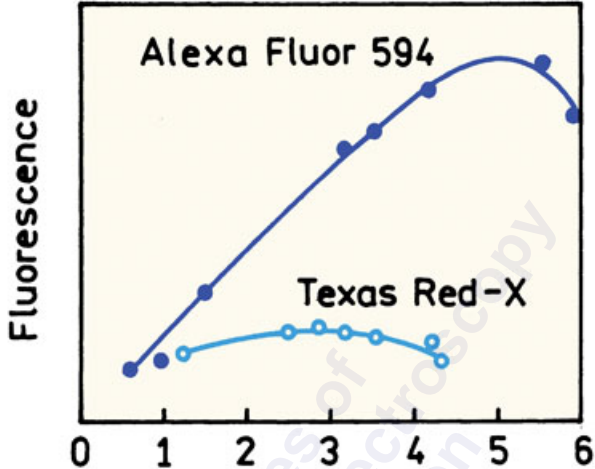


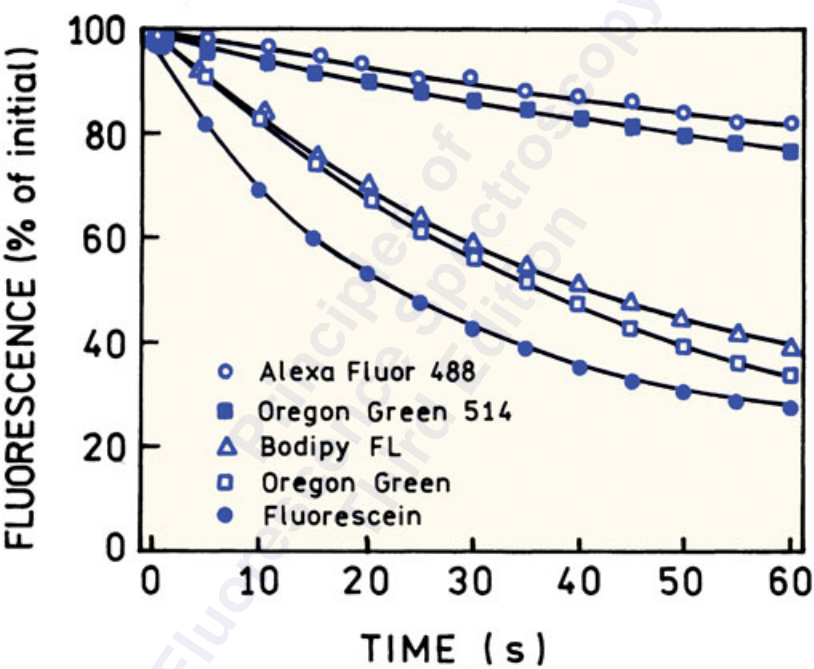
BODIPY- 581 / 591

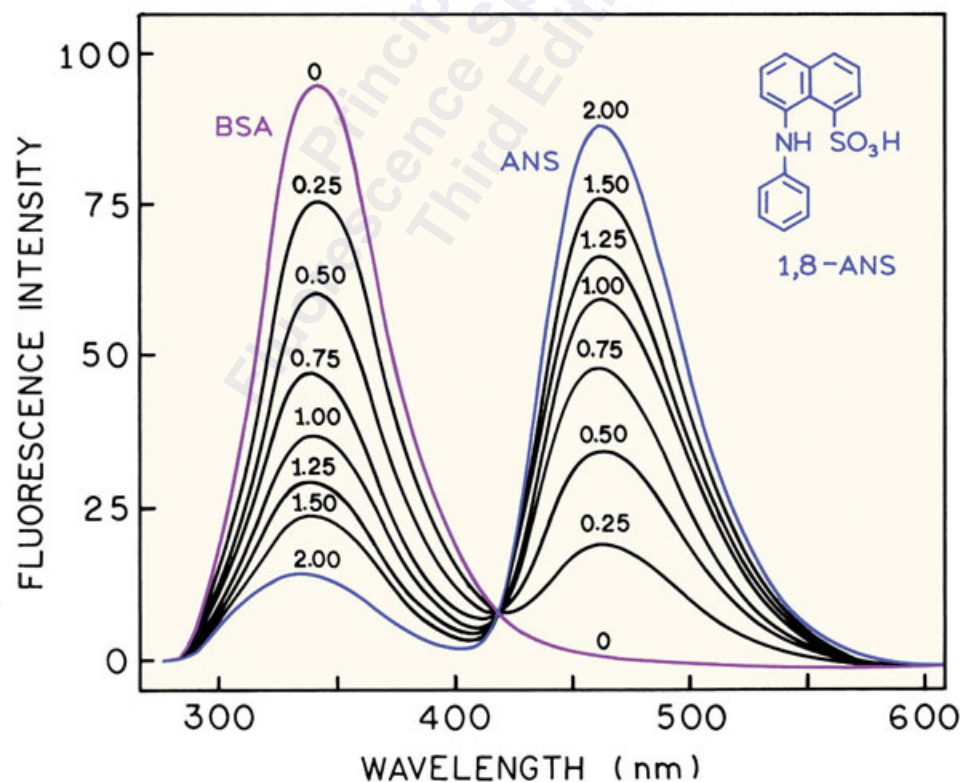
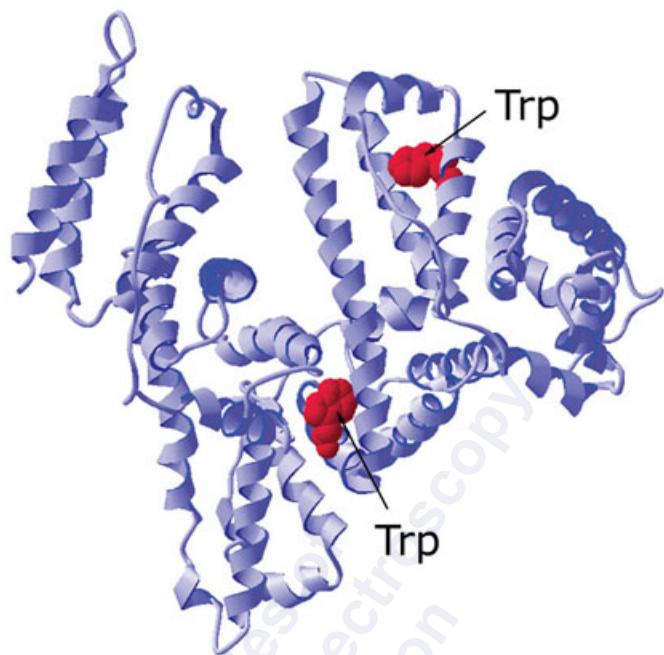


BODIPY- Texas Red

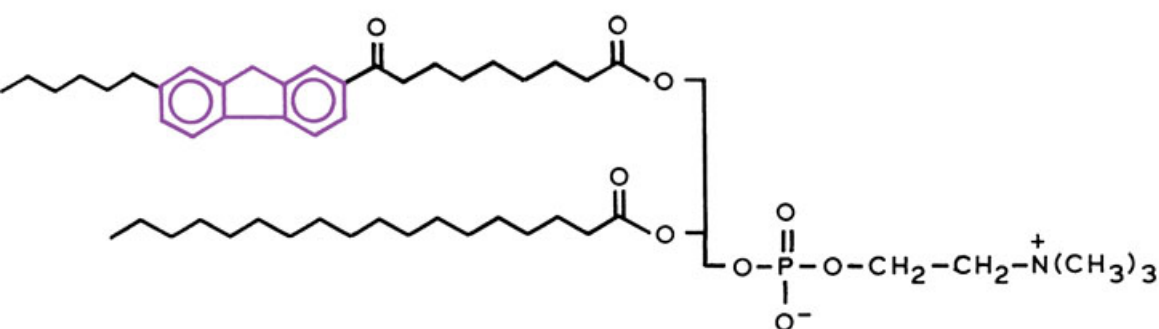
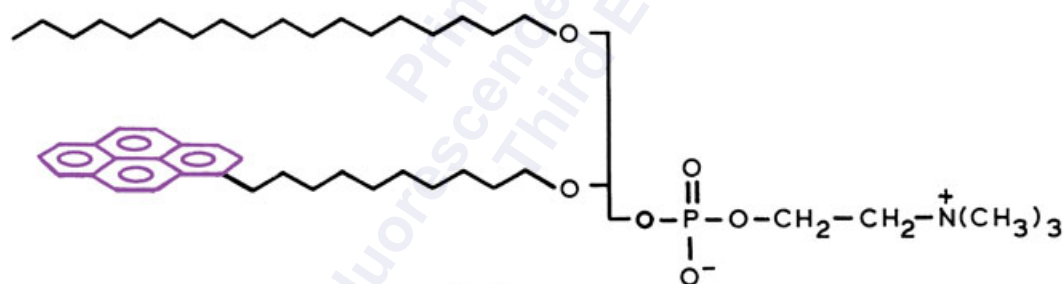
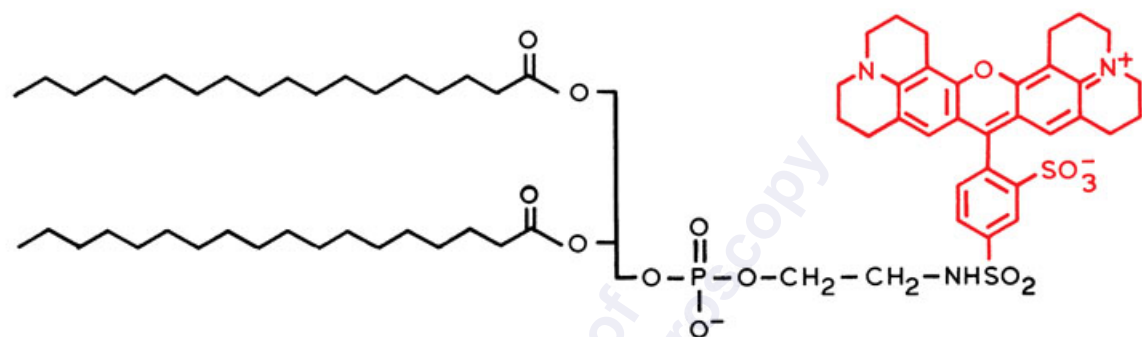
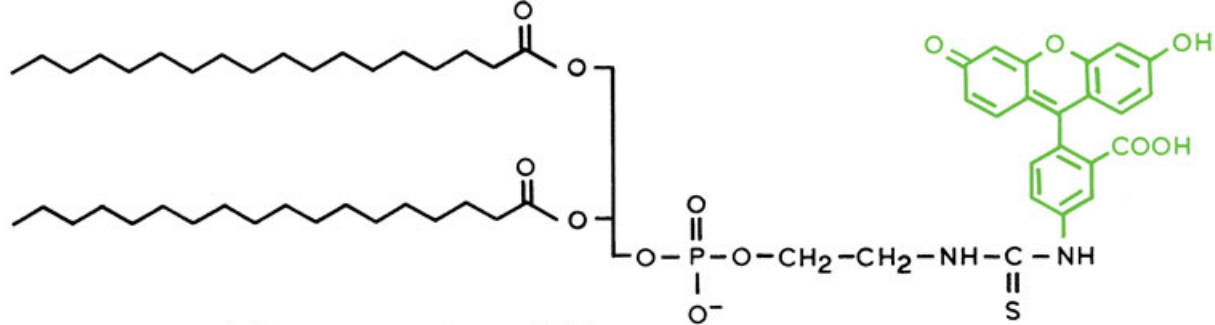


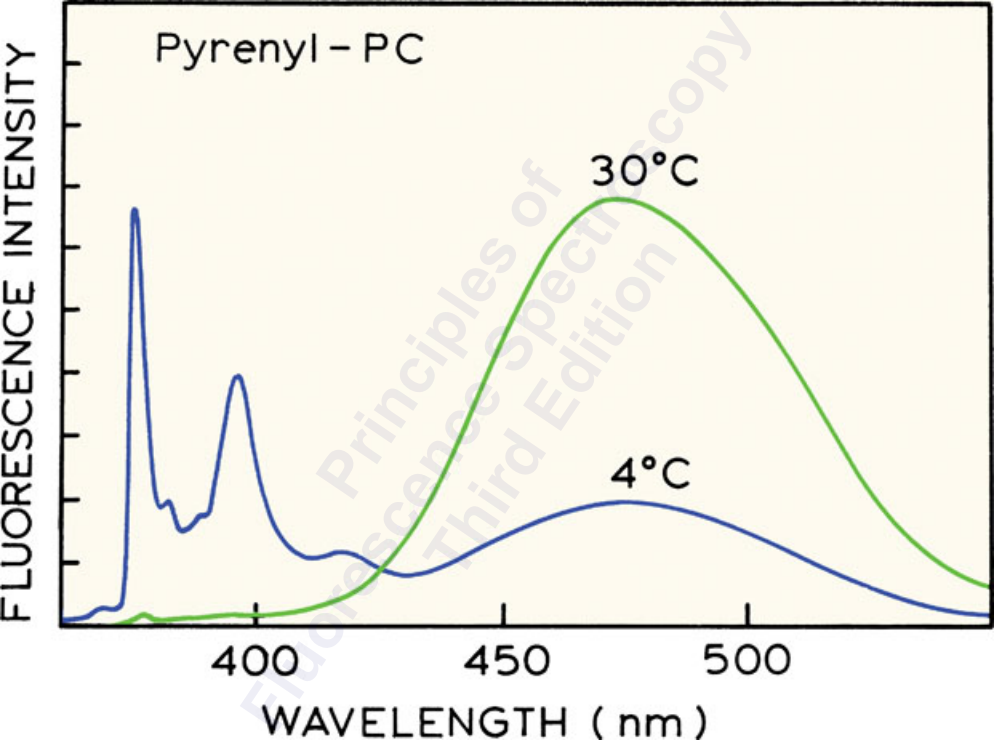


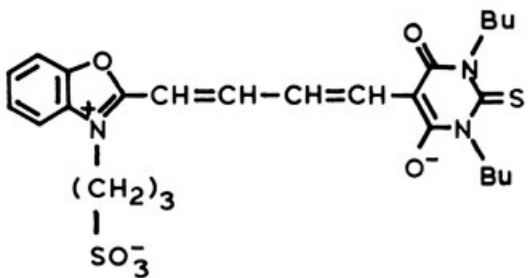




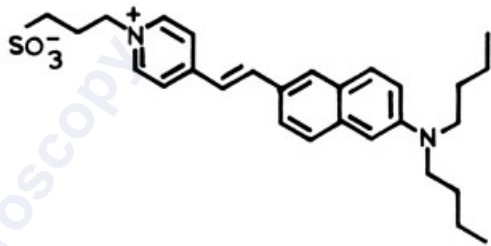




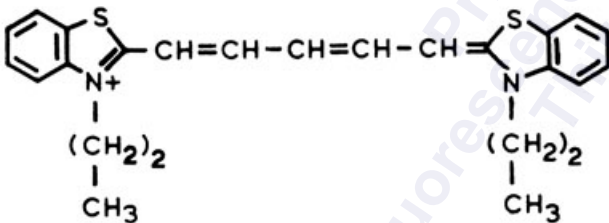




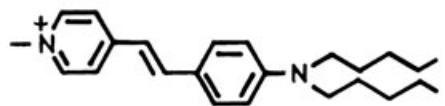
Merocyanine 540



Naphthyl Styryl



diS-C<sub>3</sub>-(5)

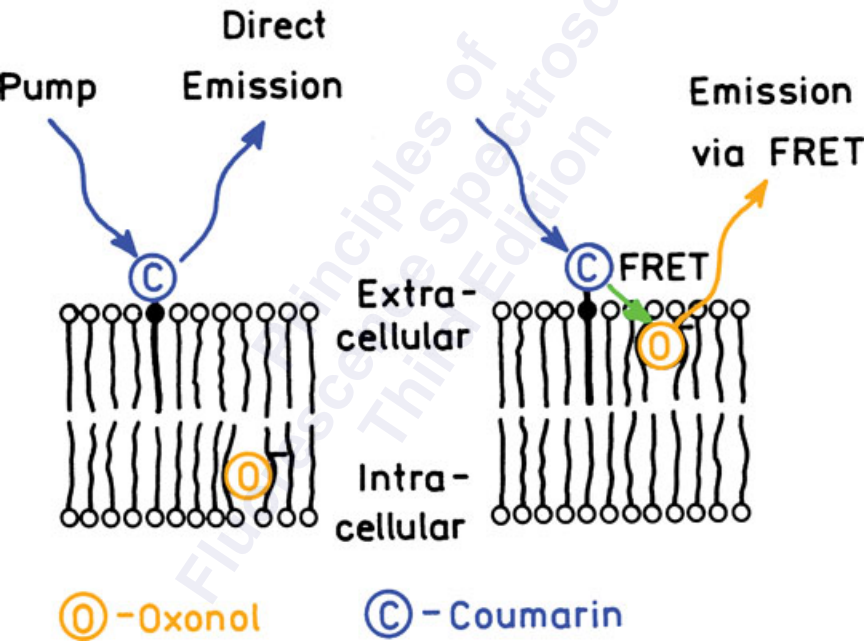


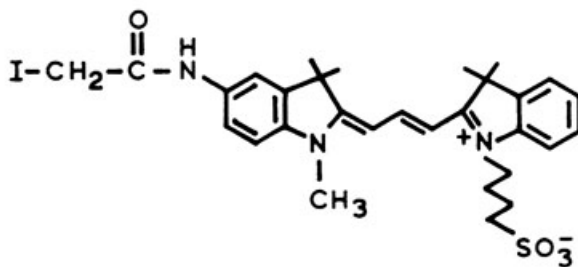
di-5-ASP



Depolarized  
( $V \gg 0$ )

Hyperpolarized  
( $V \ll 0$ )

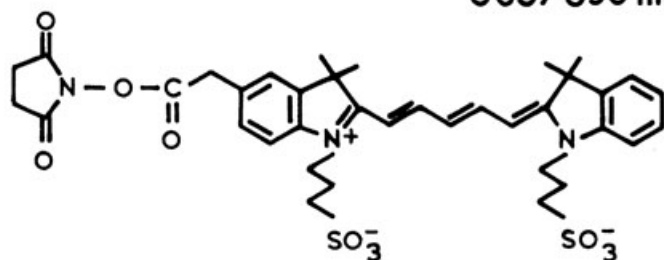


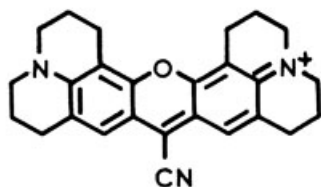


Cy-3 Iodo Acetamide

565/590 nm

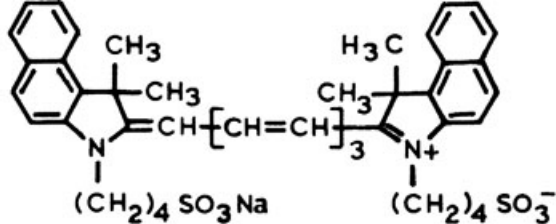
QY≈0.07





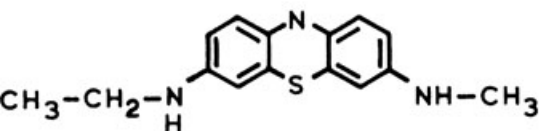
**Rhodamine 800**

680 / 700 nm



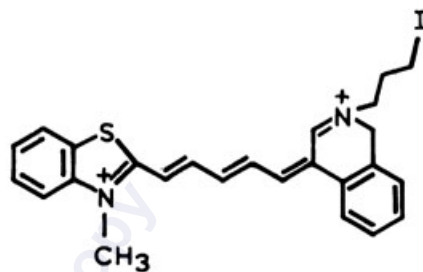
**IR-125**

782 / 830 nm



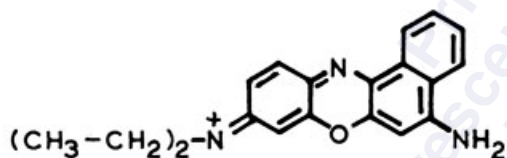
**Azure B**

638 / 660 nm



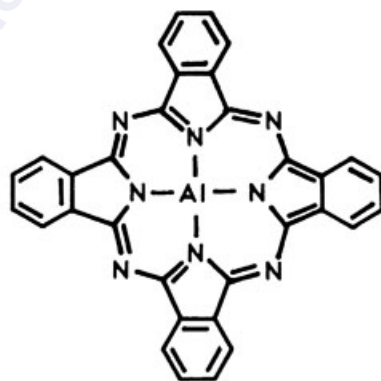
**Thiazole Orange**

735 / 765 nm



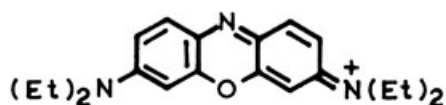
**Nile Blue**

638/660 nm



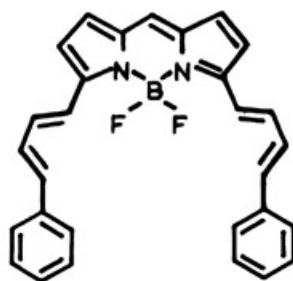
**Al Phthalocyanine**

662 / 680 nm



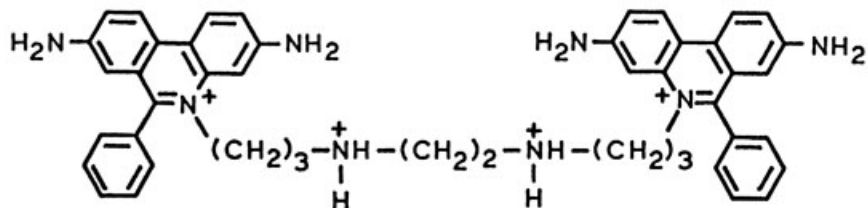
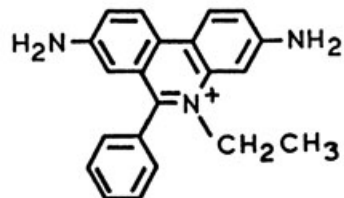
**Oxazine 1**

643 / 658 nm



**BODIPY™**

665 / 676 nm

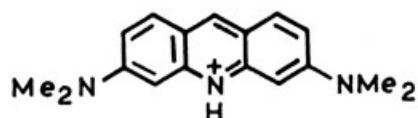


**Ethidium Bromide**

518/605 nm

**Ethidium Homodimer**

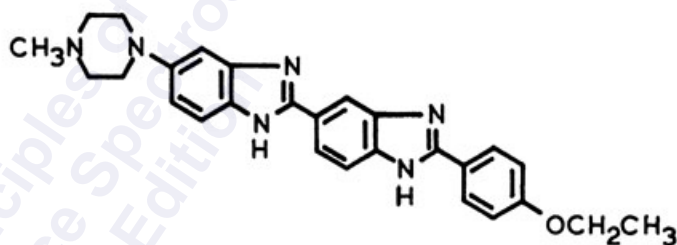
528/617 nm



**Acridine Orange**

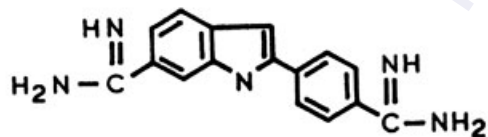
500/526 nm DNA

460/650 nm RNA



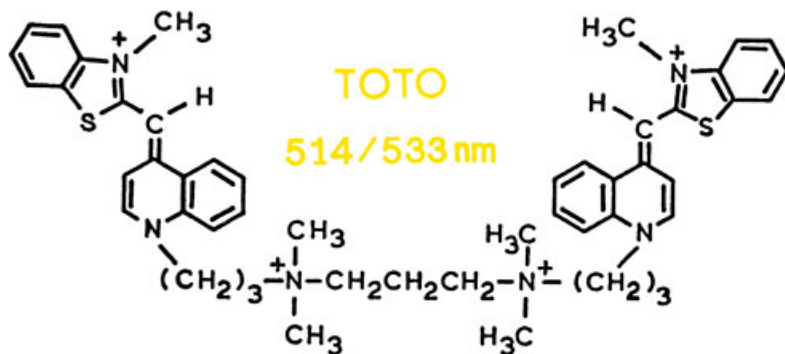
**Hoechst 33342**

350/460 nm



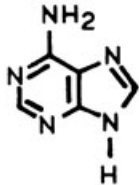
**DAPI**

355/461 nm

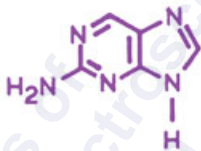


**TOTO**

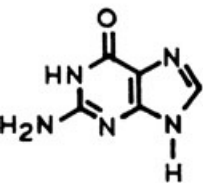
514/533 nm



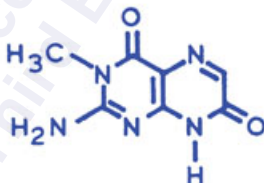
Adenine



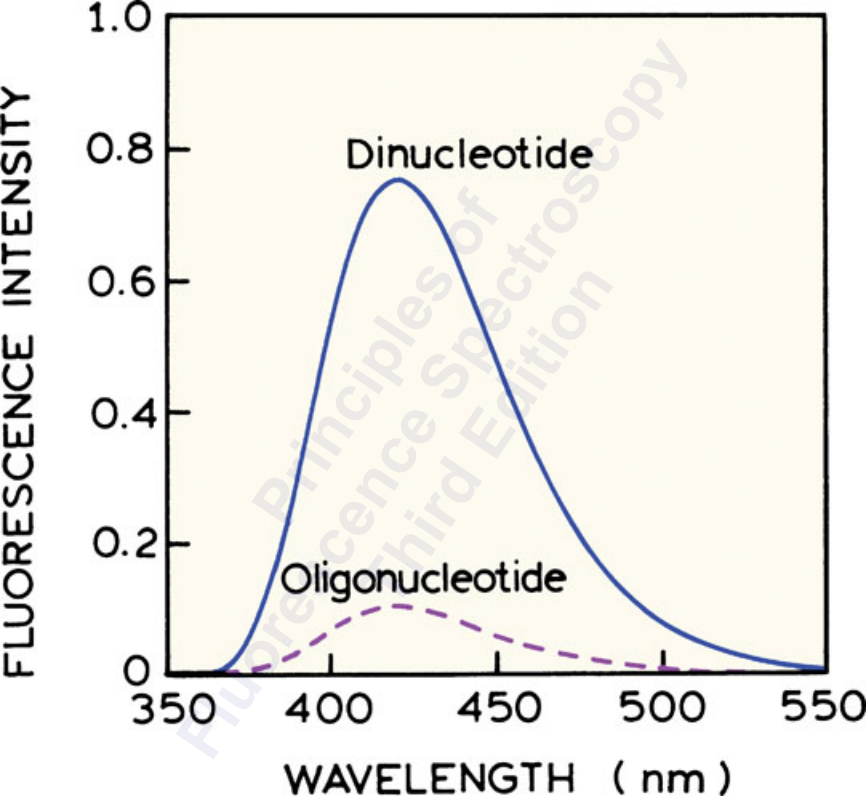
2-Aminopurine (2-AP)

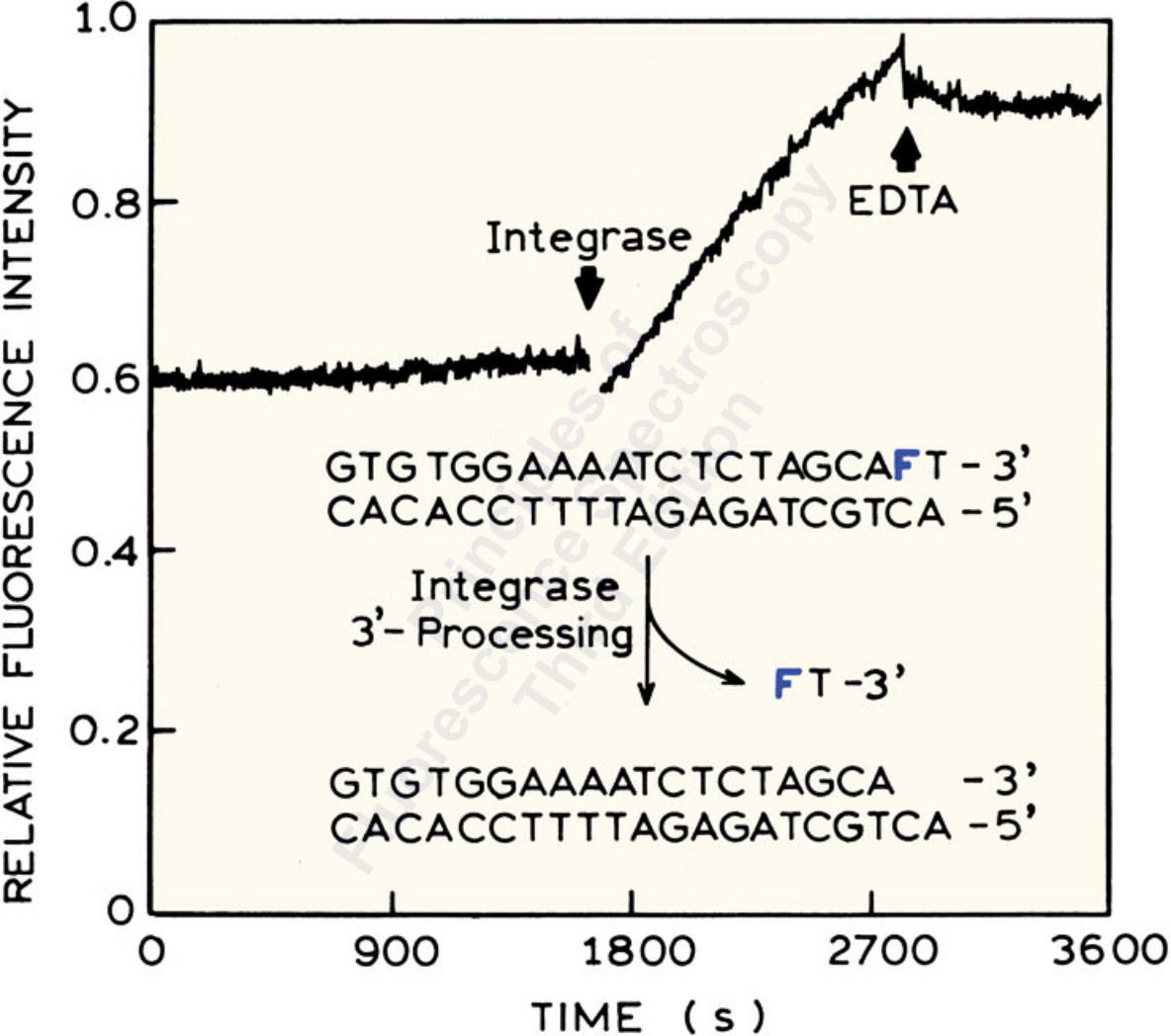


Guanine

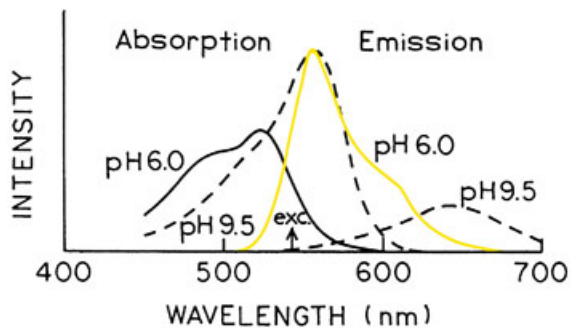
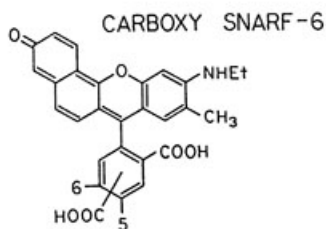
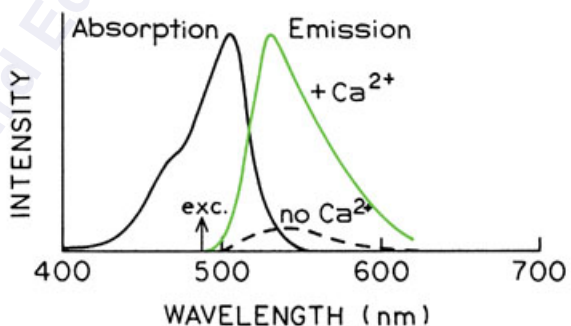
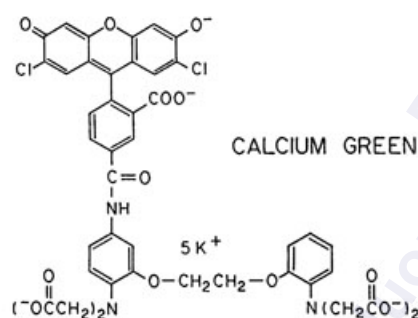
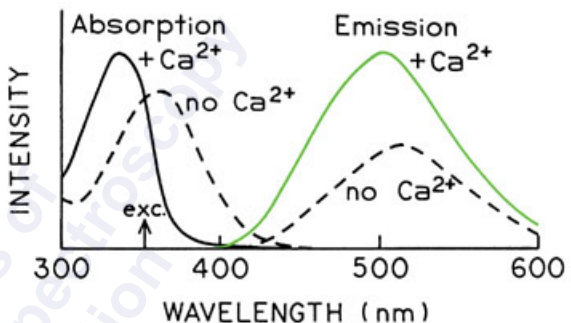
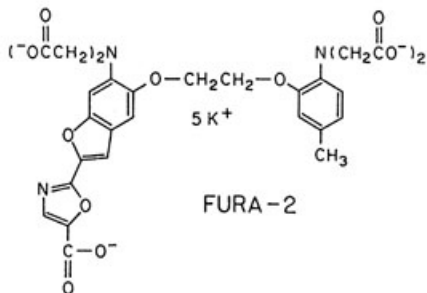
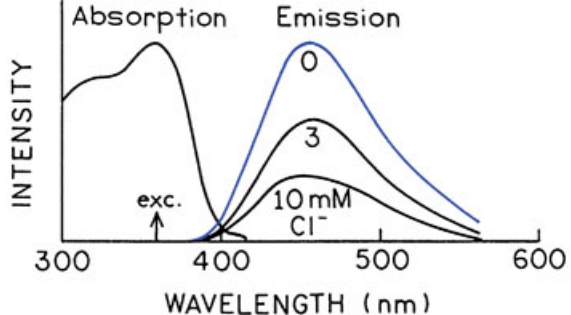
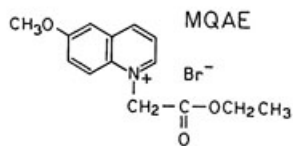


Isoxanthopterin (IXP)

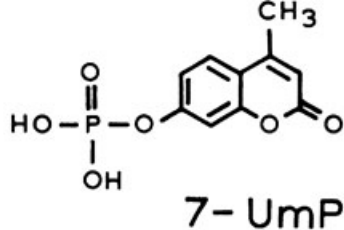




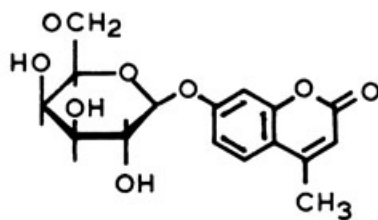
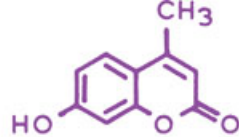




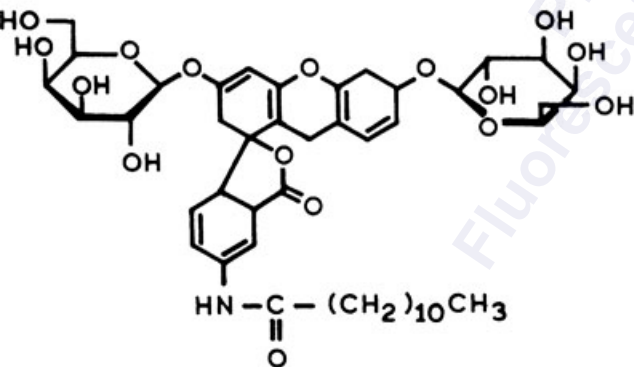
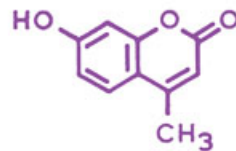




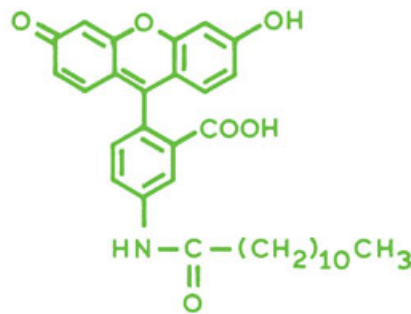
Alkaline  
Phosphatase

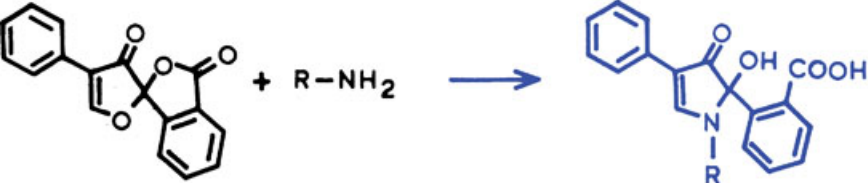


$\beta$ -galactosidase

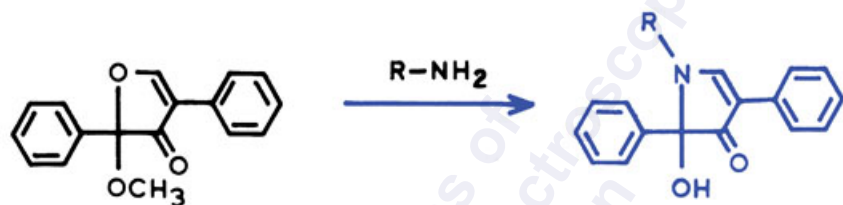


$\beta$ -galactosidase

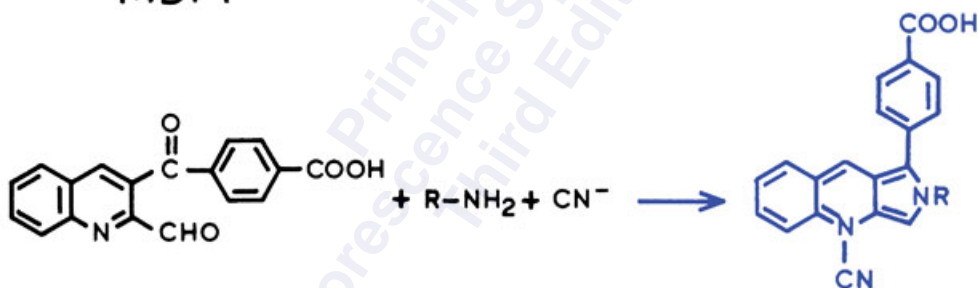




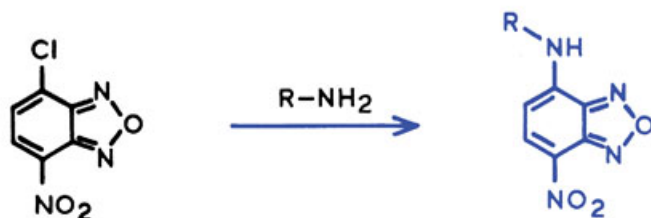
Fluorescamine



MDPF

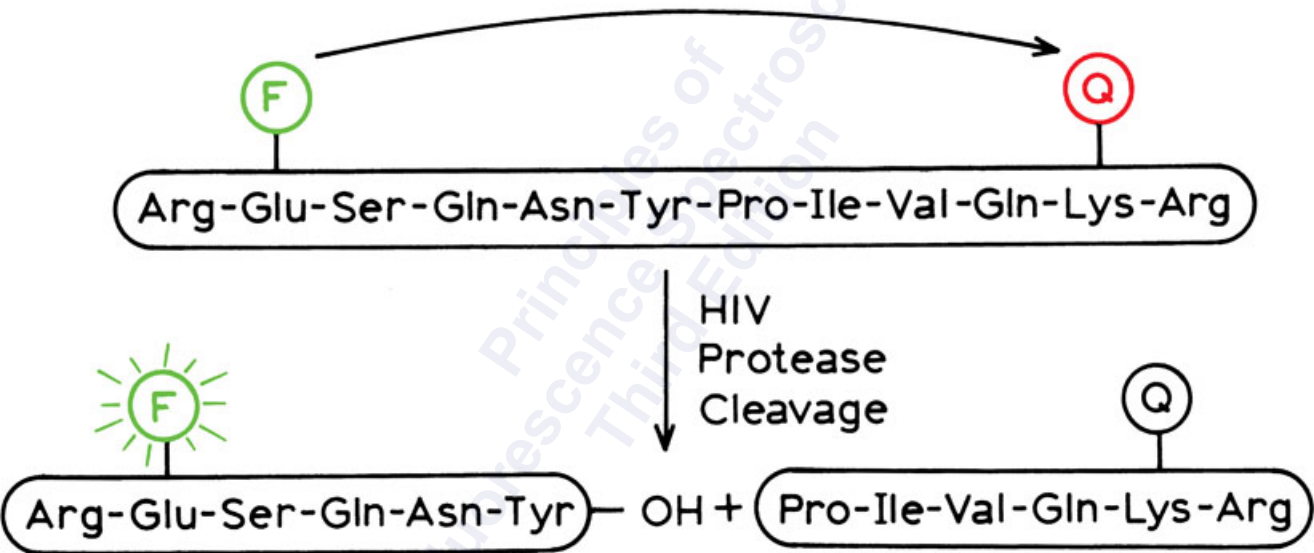


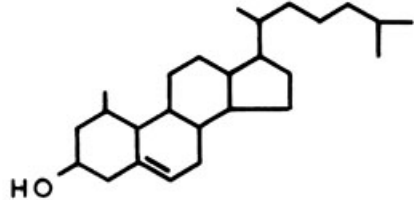
CBQCA



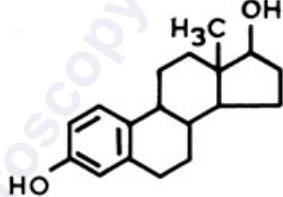
NBD-Cl

# Resonance Energy Transfer

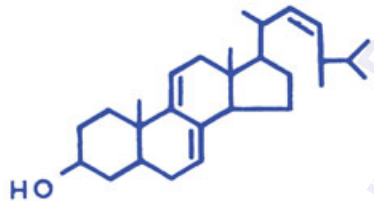




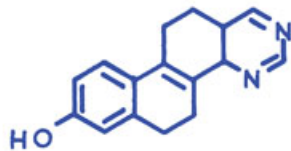
Cholesterol



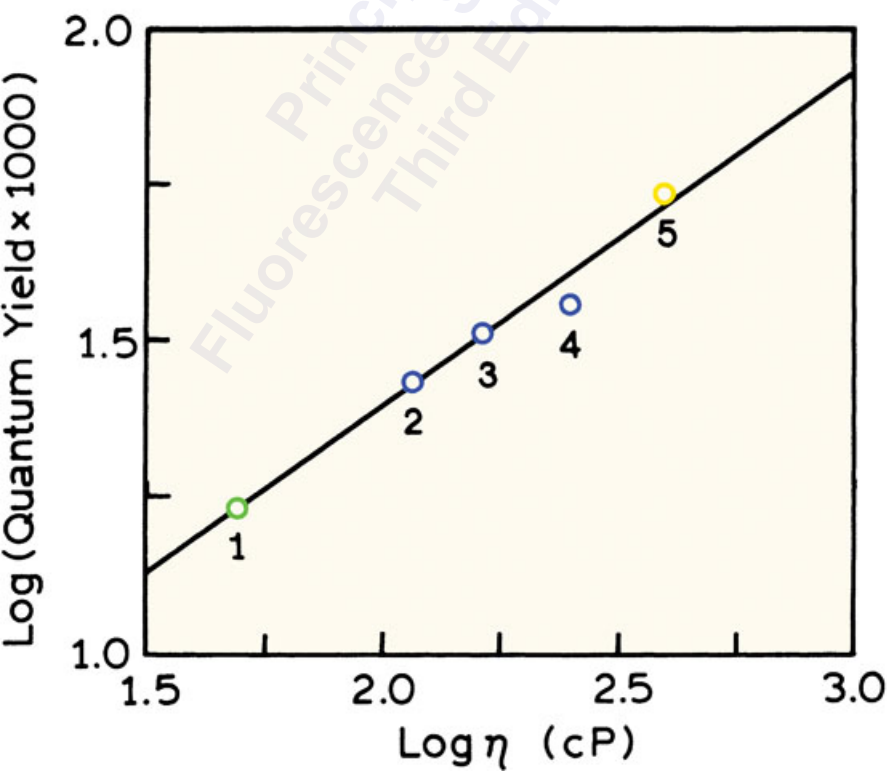
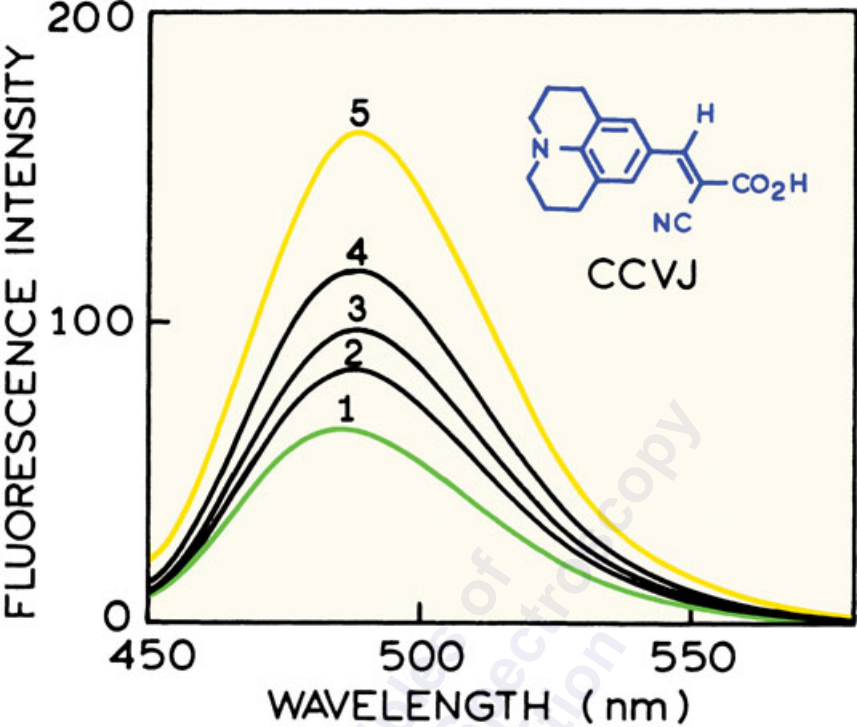
Estradiol

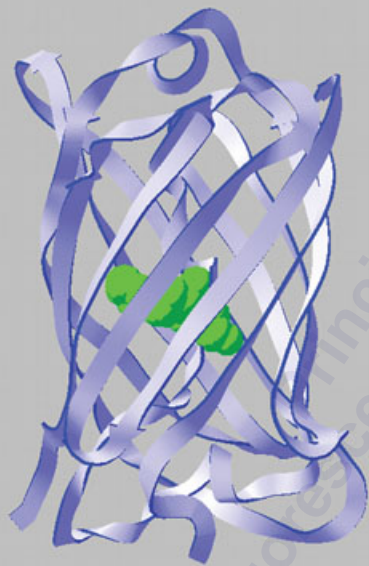


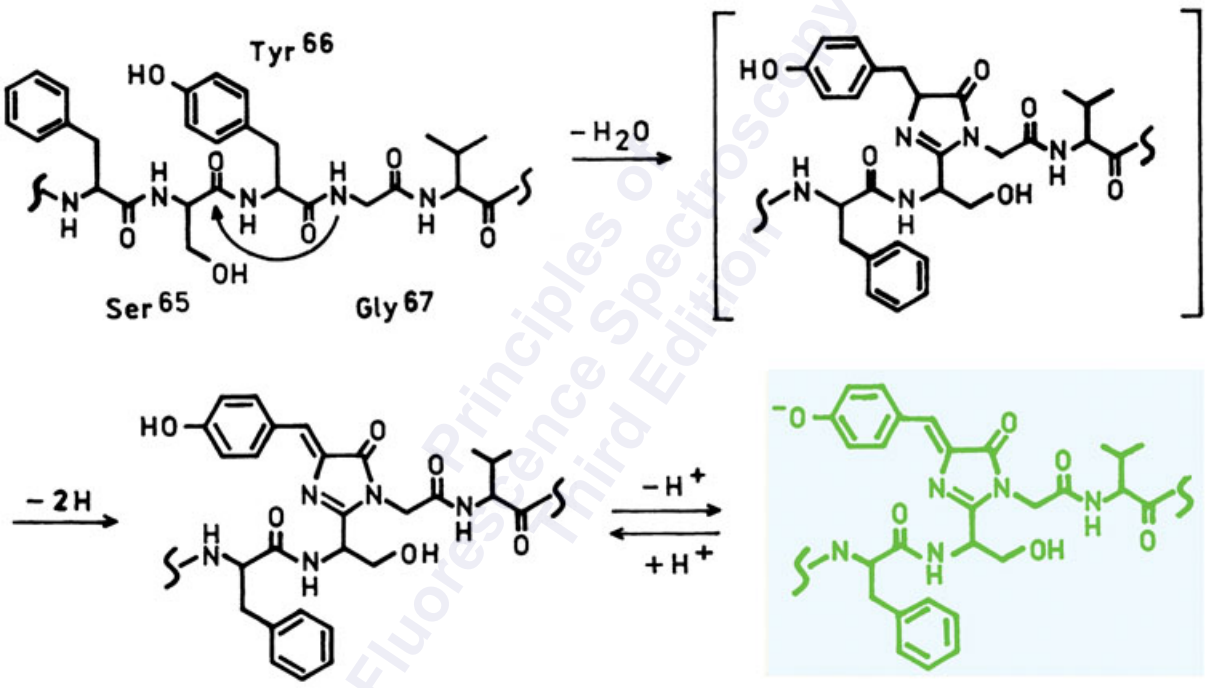
Dehydroergosterol



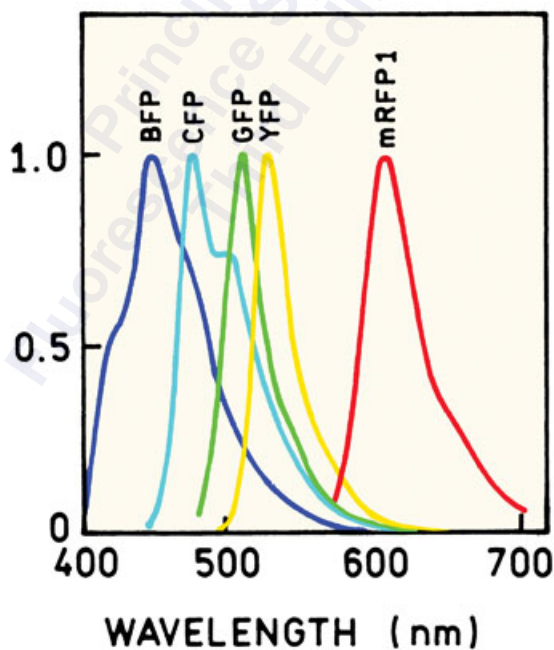
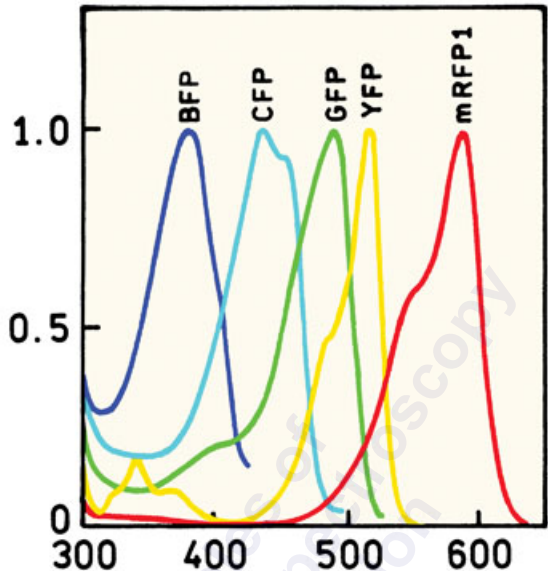
Azatetrahydrochrysene





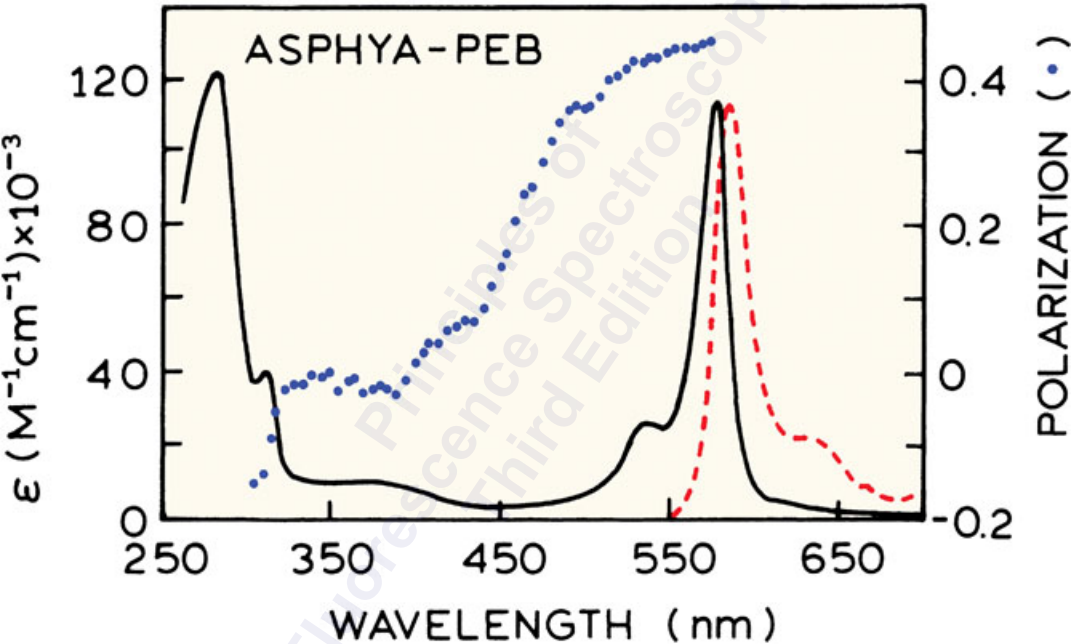


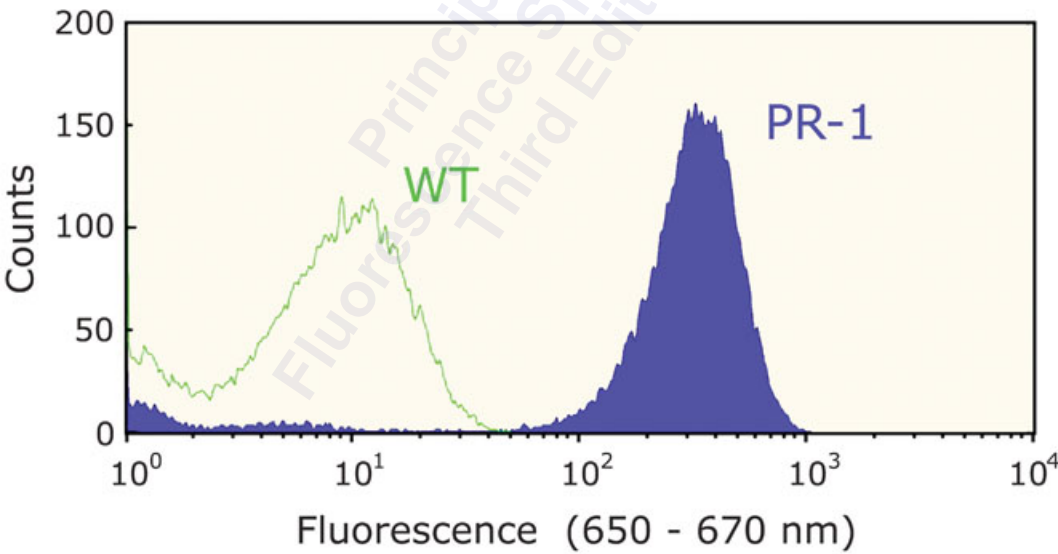
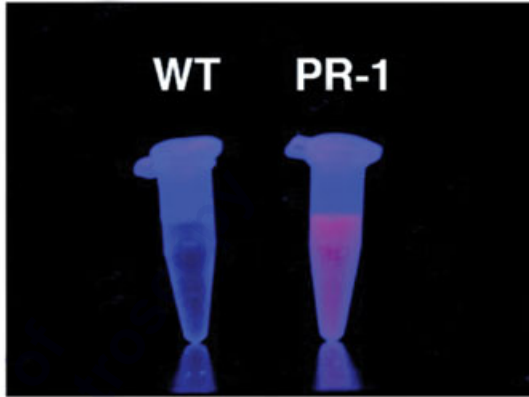
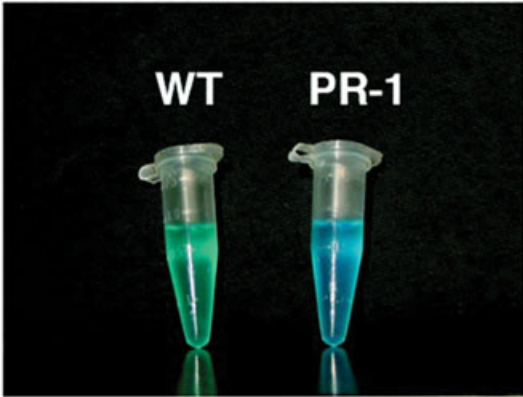
NORMALIZED FLUORESCENCE

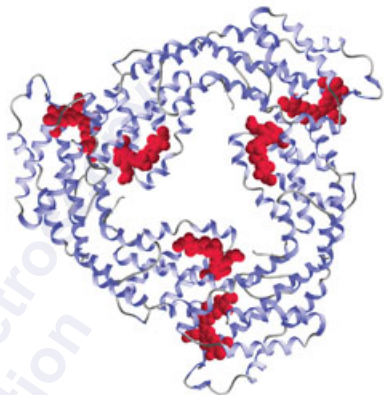
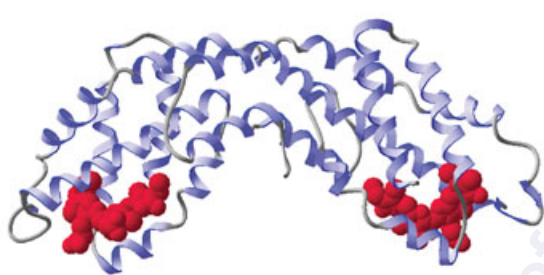




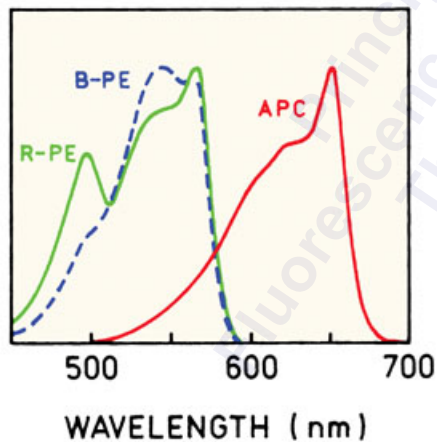




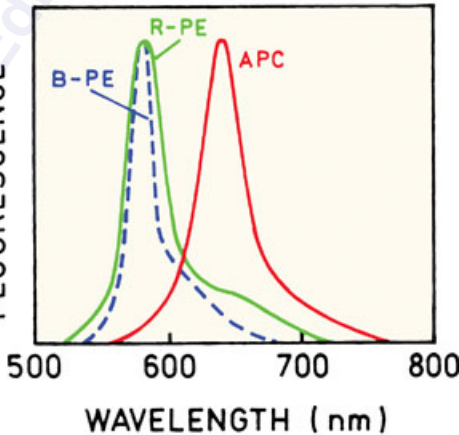




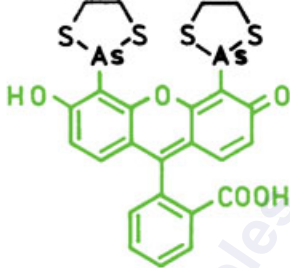
ABSORPTION



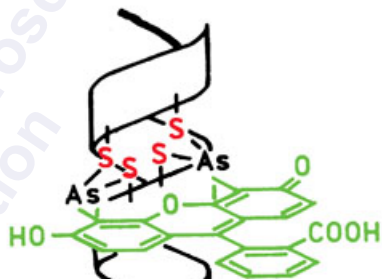
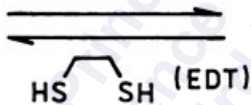
FLUORESCENCE



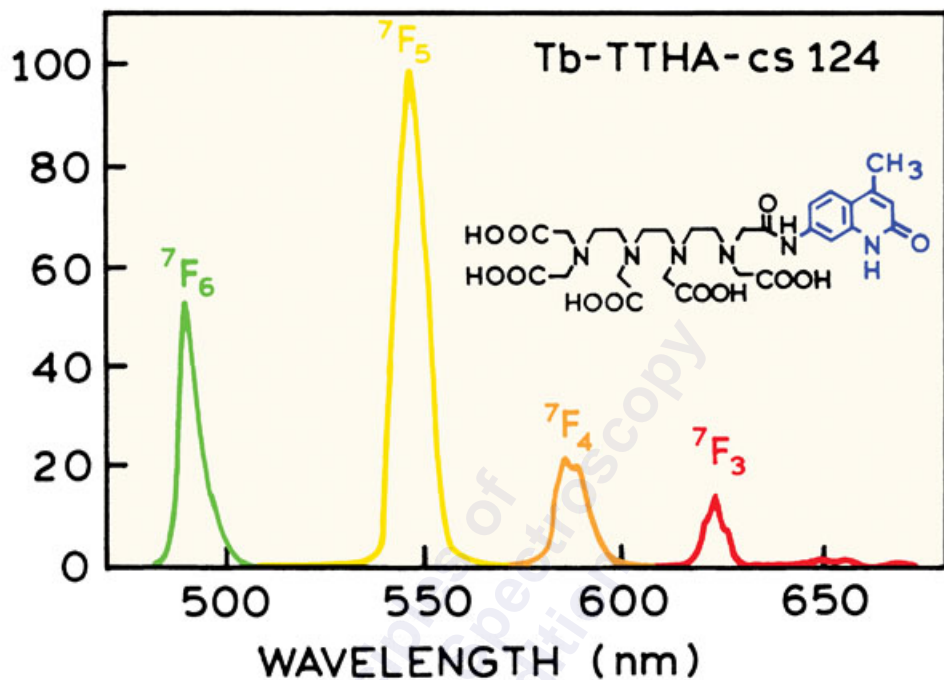
$\alpha$ -helix containing  
a 4Cys-motif



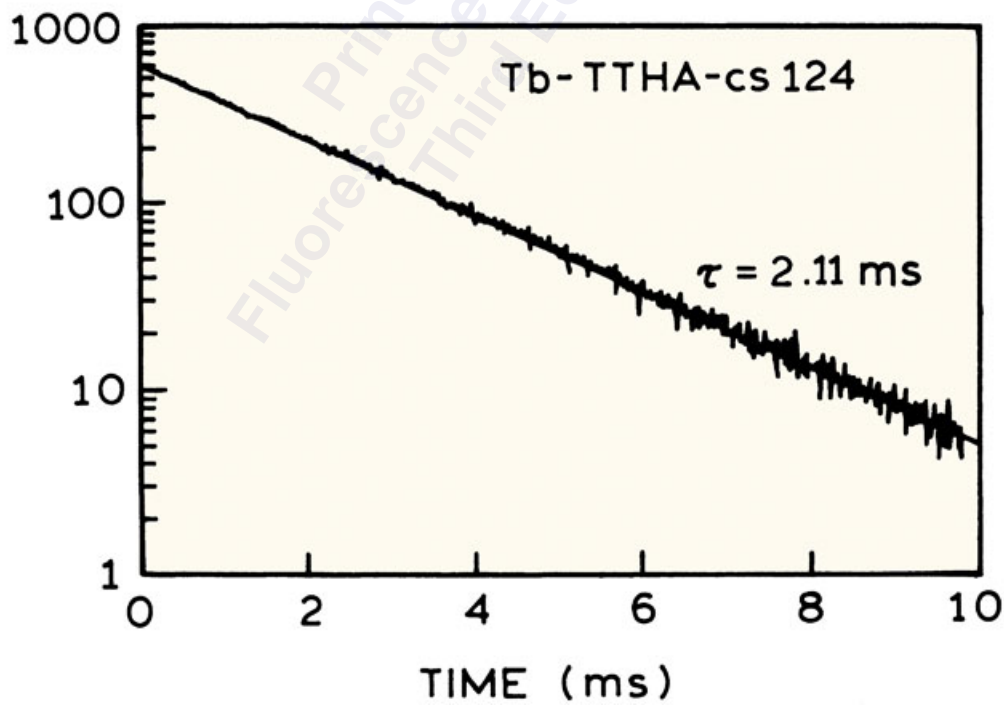
FLASH-EDT<sub>2</sub>

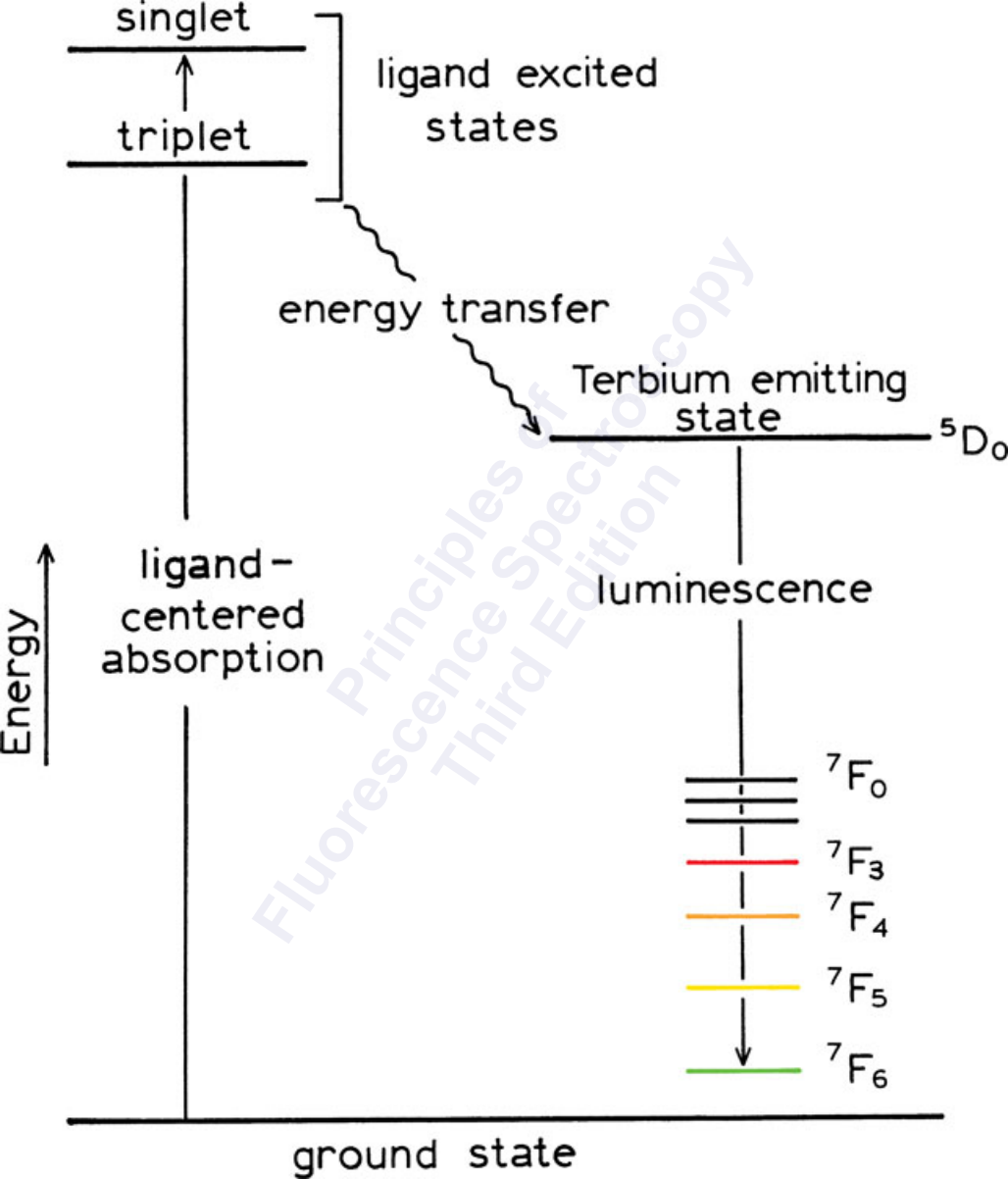


TERBIUM EMISSION

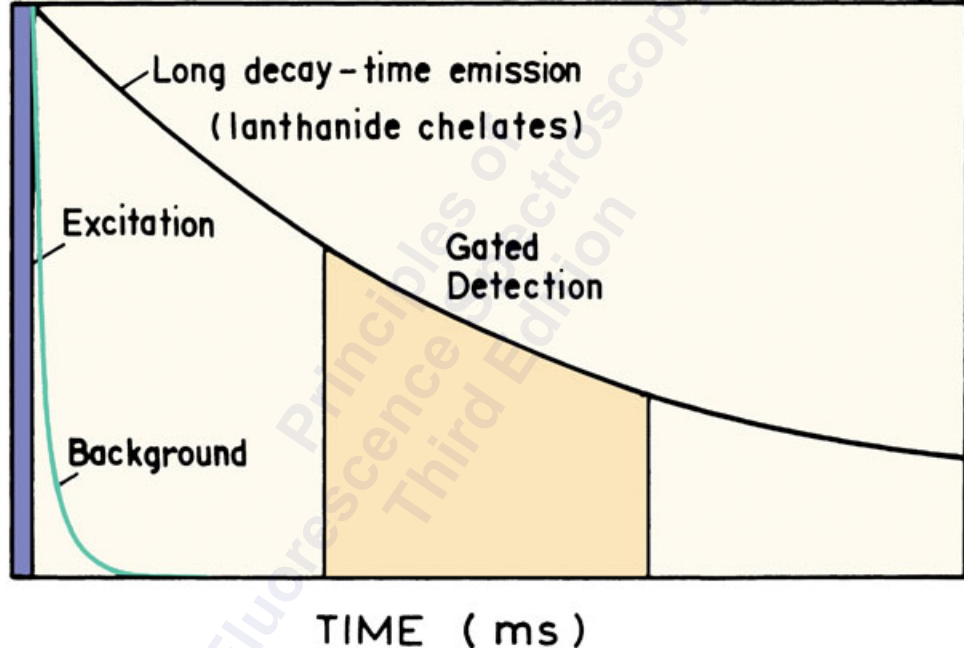


TERBIUM EMISSION





FLUORESCENCE





INTENSITY

10000

8000

6000

4000

$\text{Ru}(\text{bpy})_2(\text{mcbpy})$  -PE in DPPG Vesicles

5°C

$\tau$  (ns)

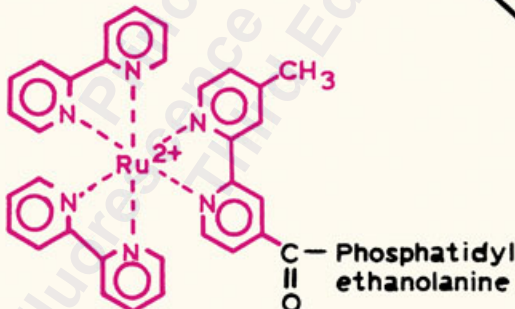
20

606

46

480

369



TIME (ns)

0

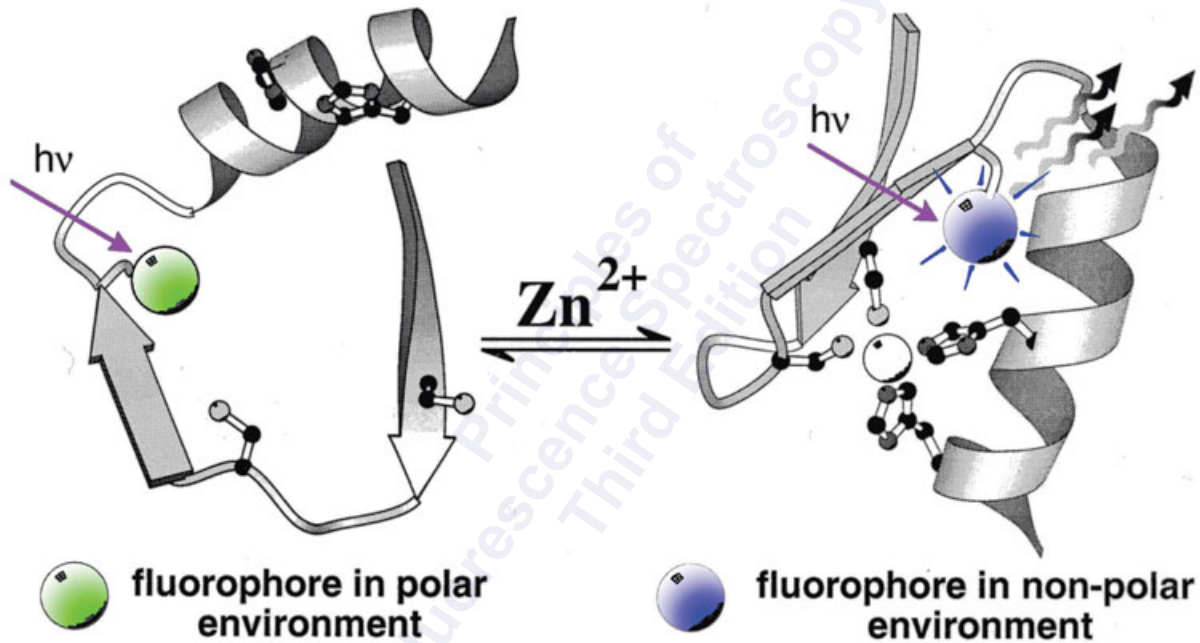
50

100

150

200

250



RELATIVE FLUORESCENCE

1.0

[Zn] ( $\mu\text{M}$ )

2.0

1.5

1.0

0.8

0.5

0.3

0.1

0

$I/I_0(475\text{ nm})$

5

3

1

[Zn] ( $\mu\text{M}$ )

0 0.2 0.4 0.6 0.8 1.0

Exc. = 333 nm

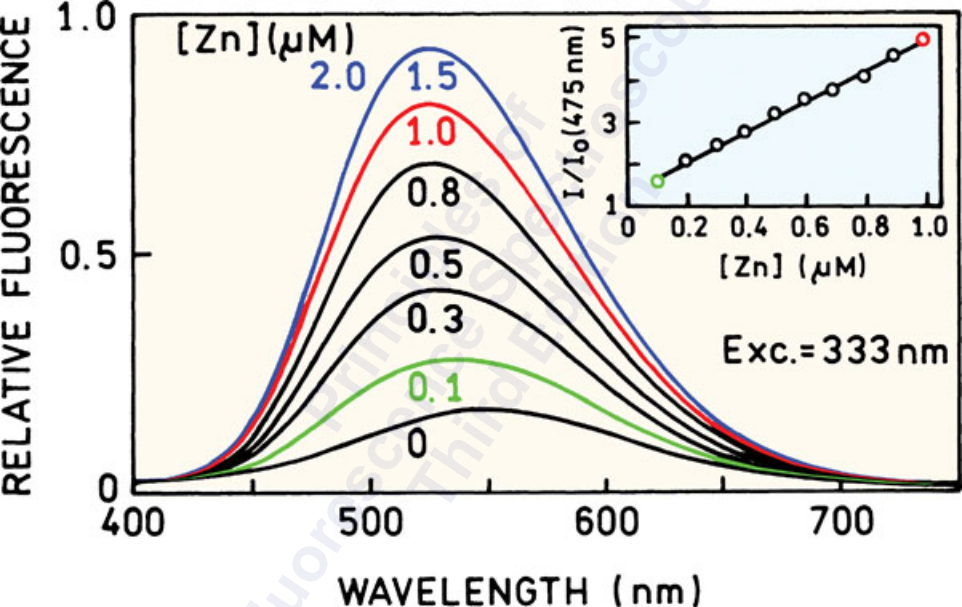
400

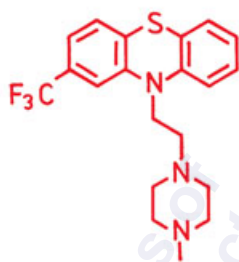
500

600

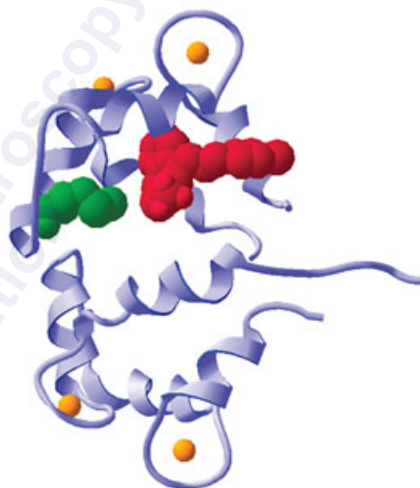
700

WAVELENGTH (nm)



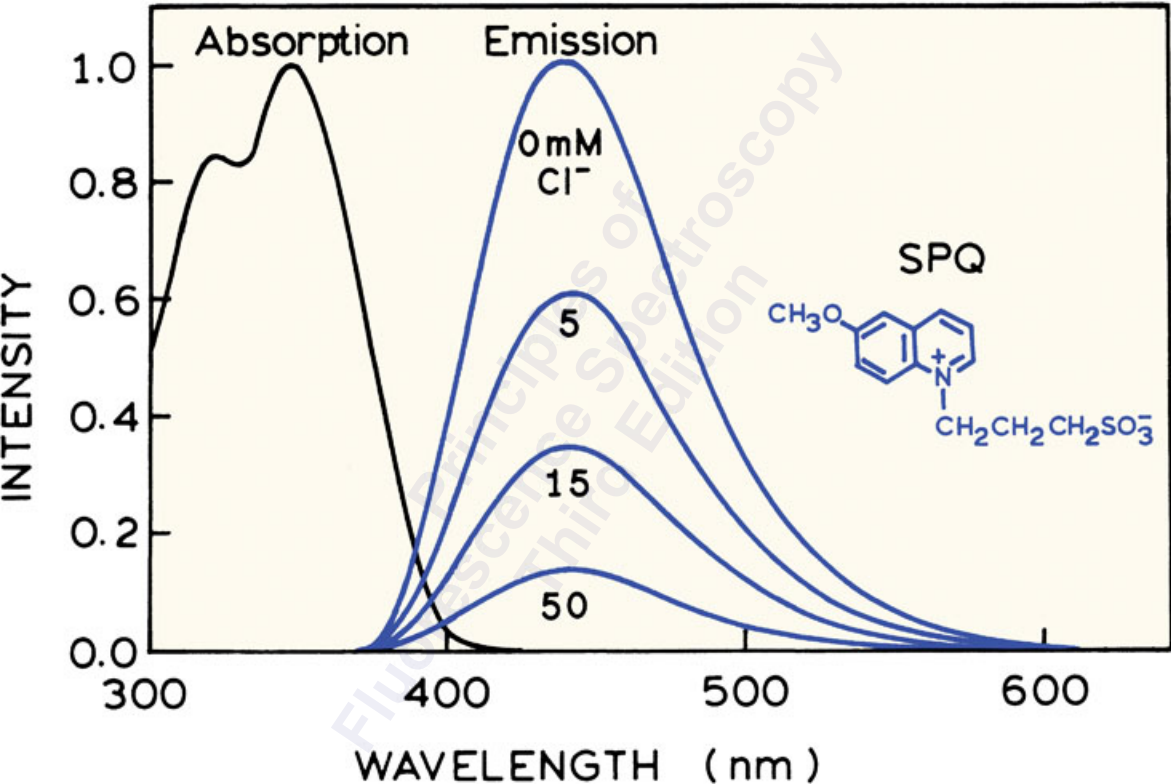


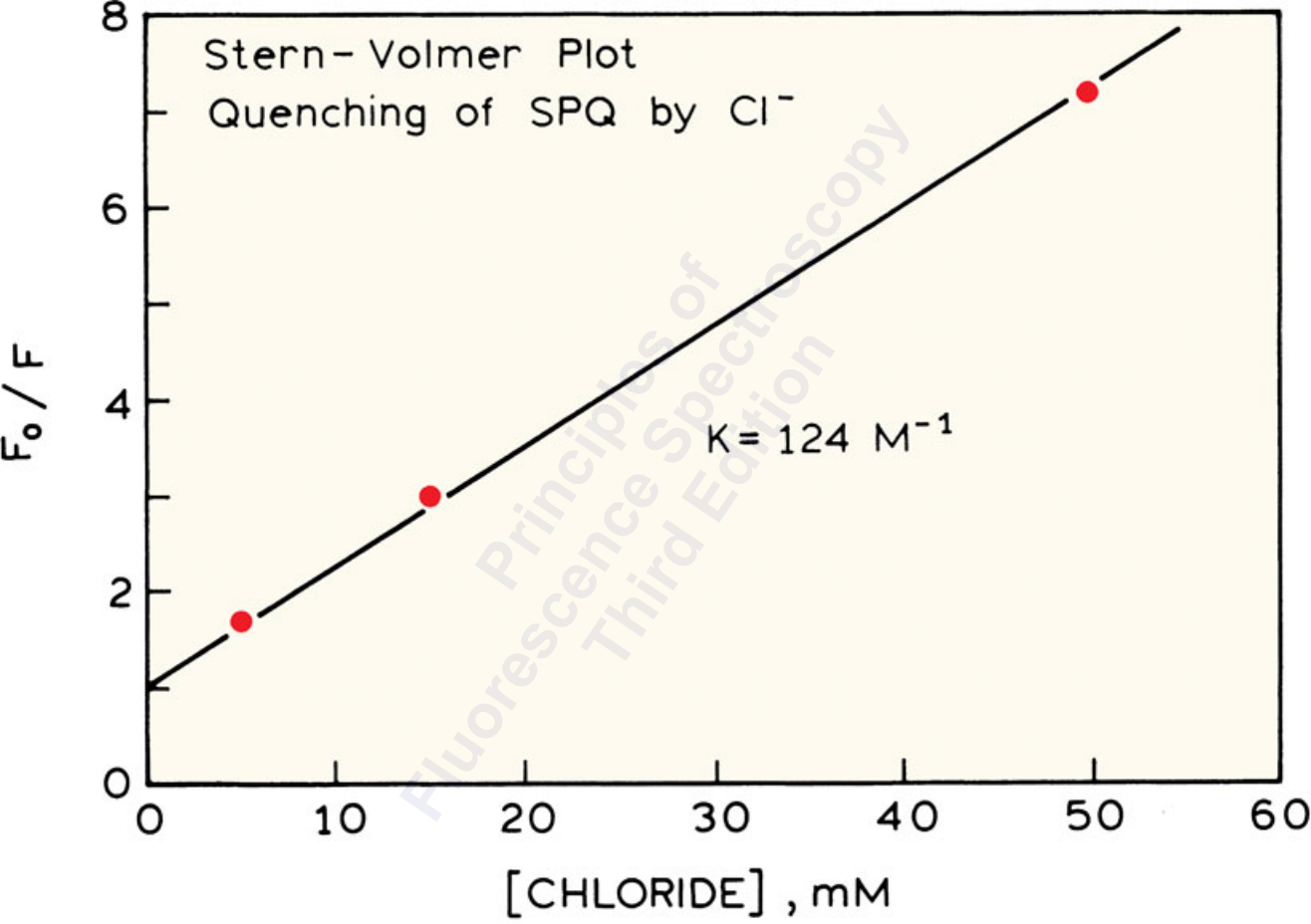
Trifluoperazine



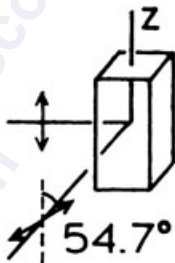
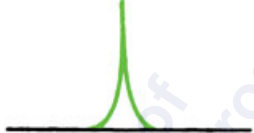
"Open Conformation"

"Bound Conformation"

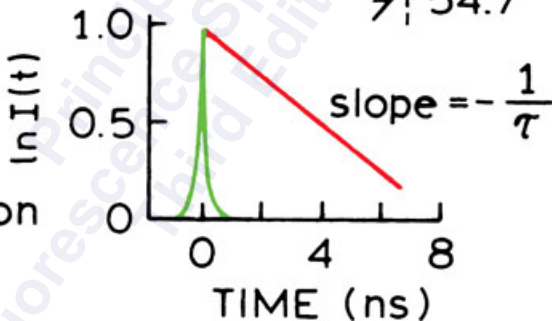




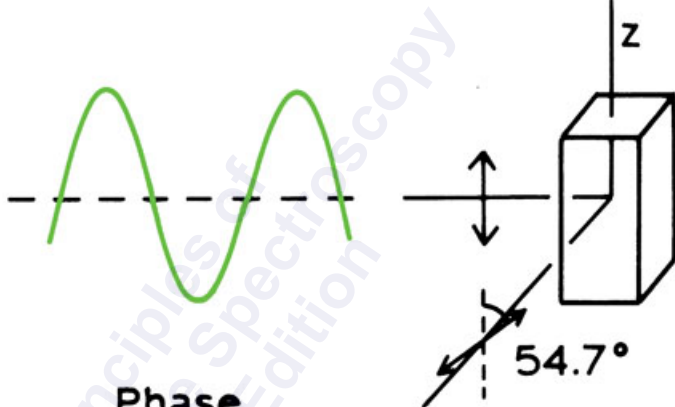
Pulsed  
Excitation



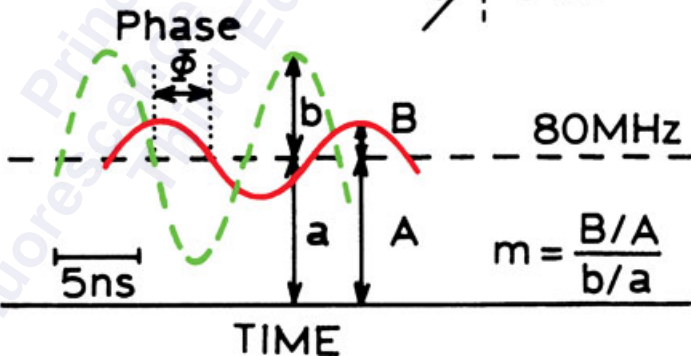
Emission



MODULATED  
EXCITATION

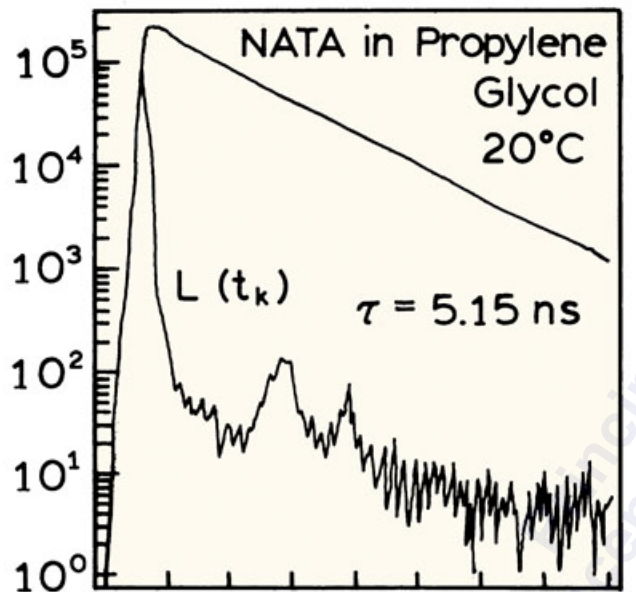


EMISSION

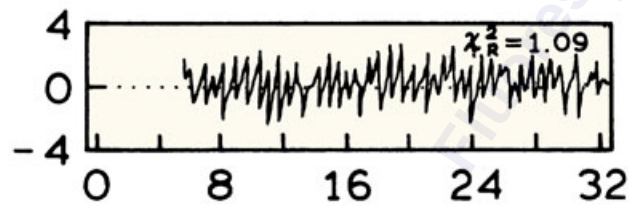




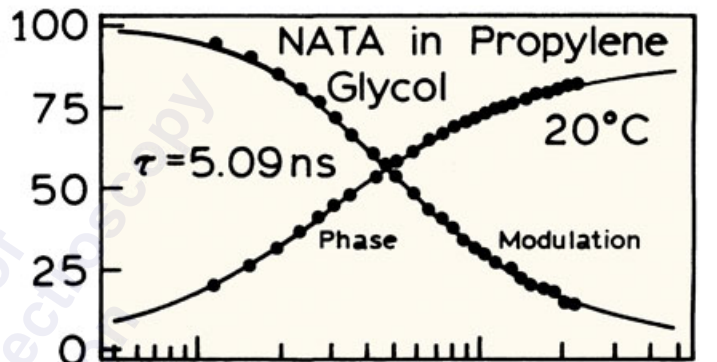
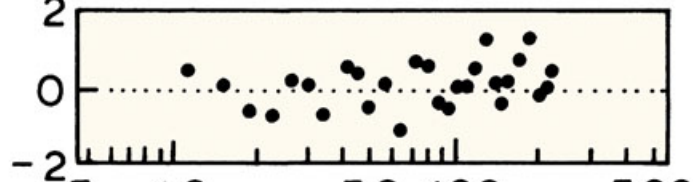
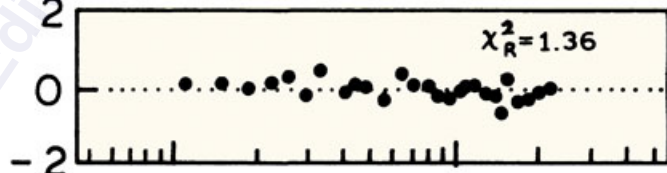
COUNTS



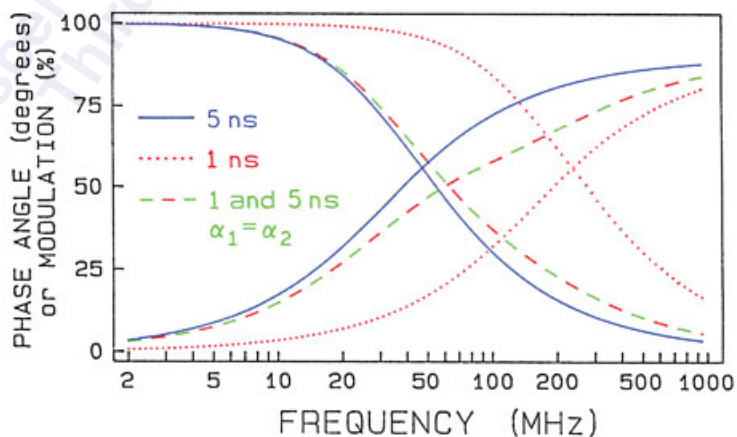
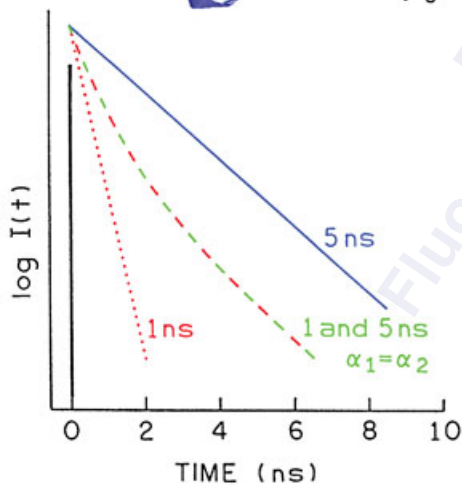
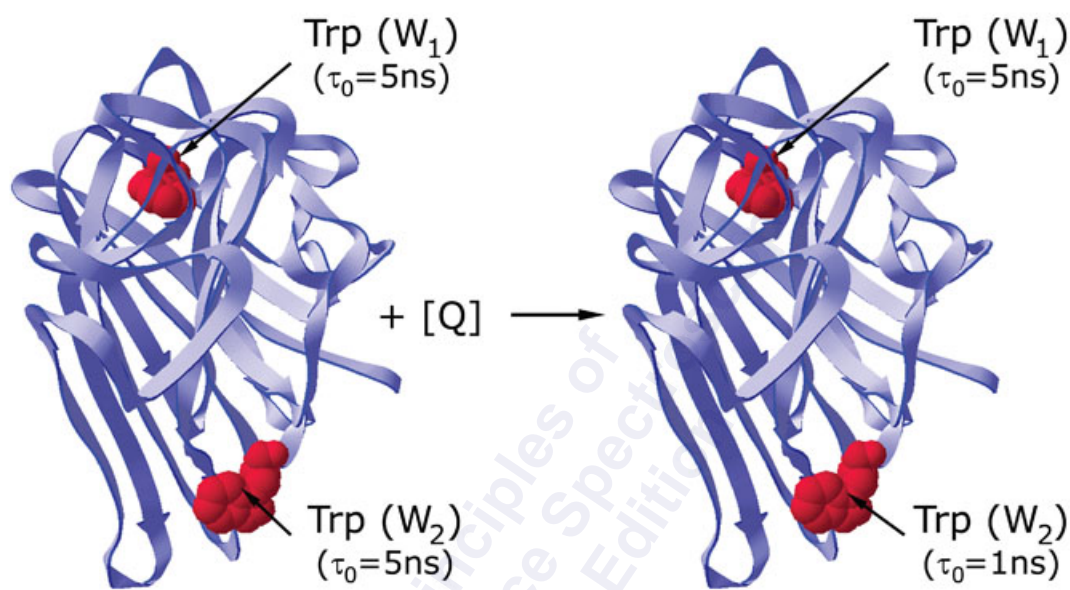
DEV.



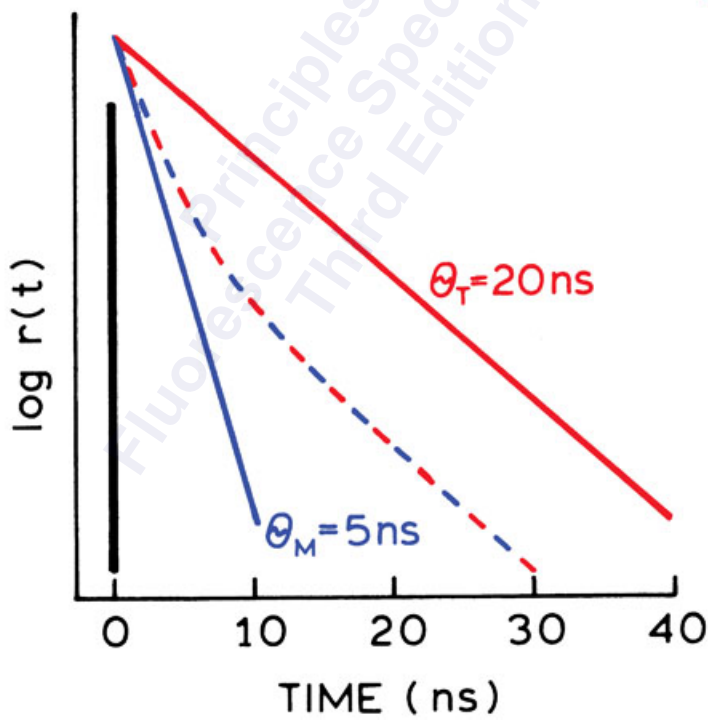
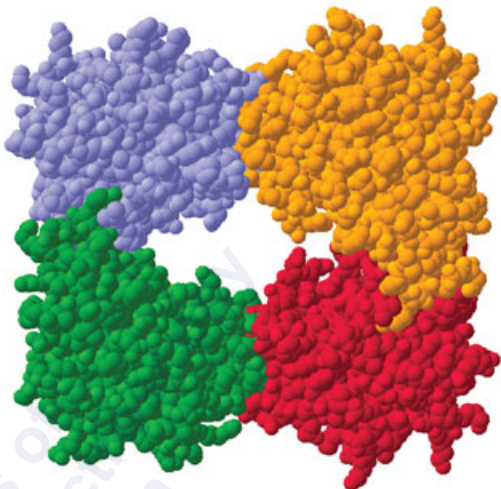
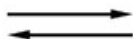
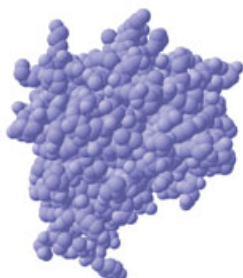
TIME (ns)

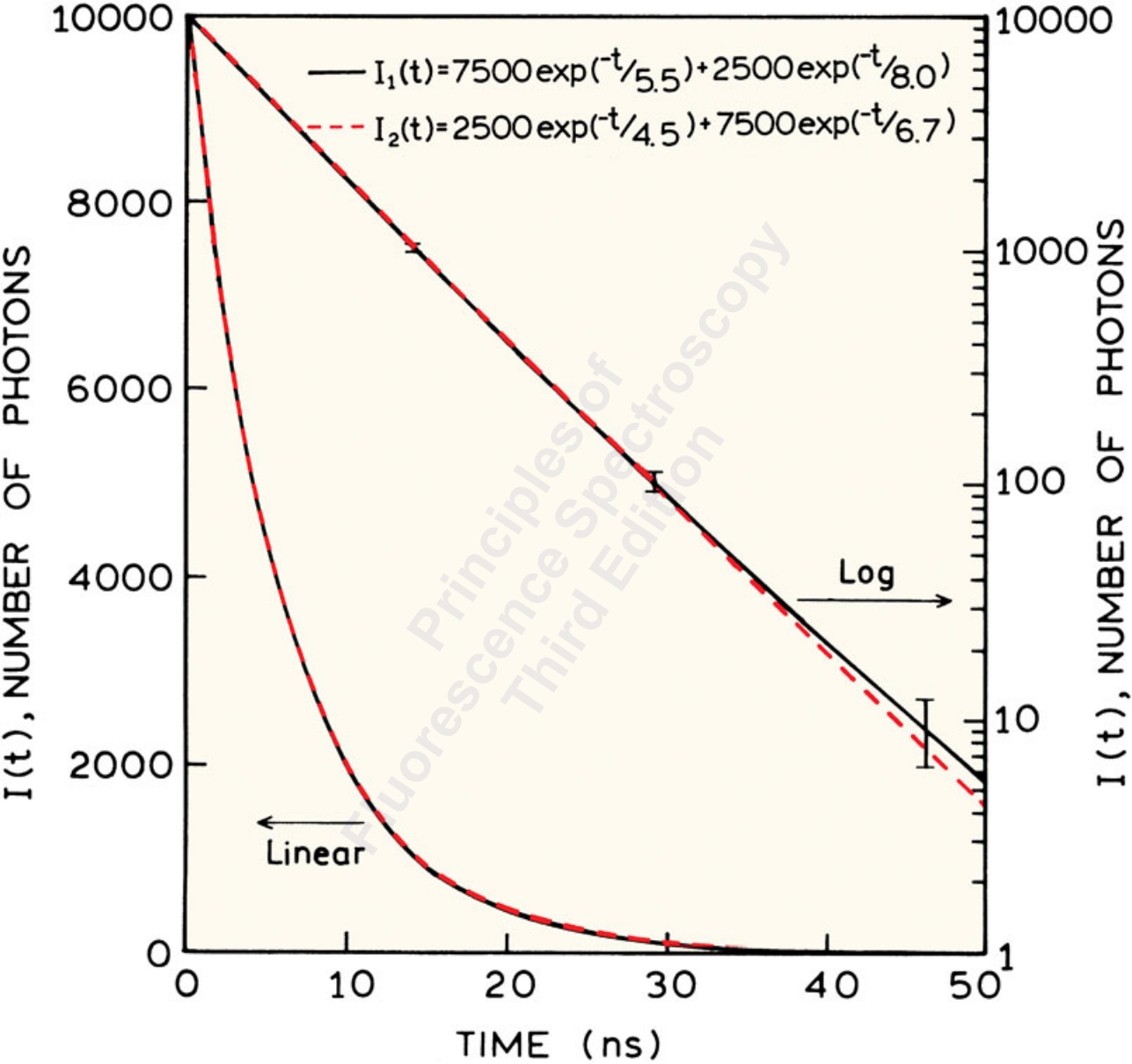
PHASE ANGLE (degrees)  
or MODULATION (%)DEVIATIONS  
MODULATION PHASE

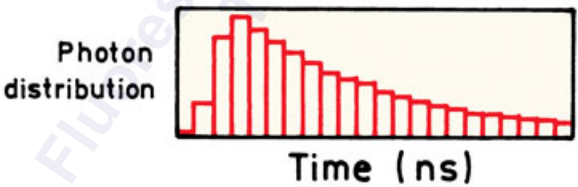
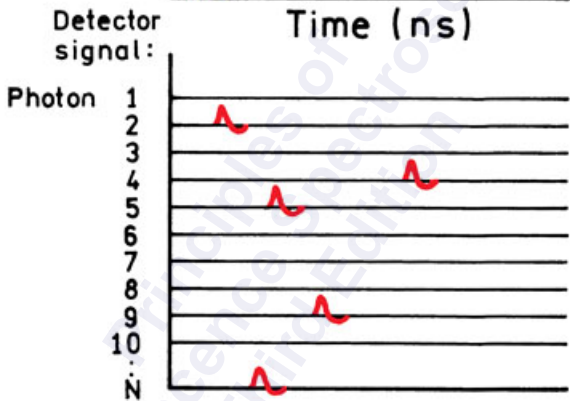
FREQUENCY (MHz)

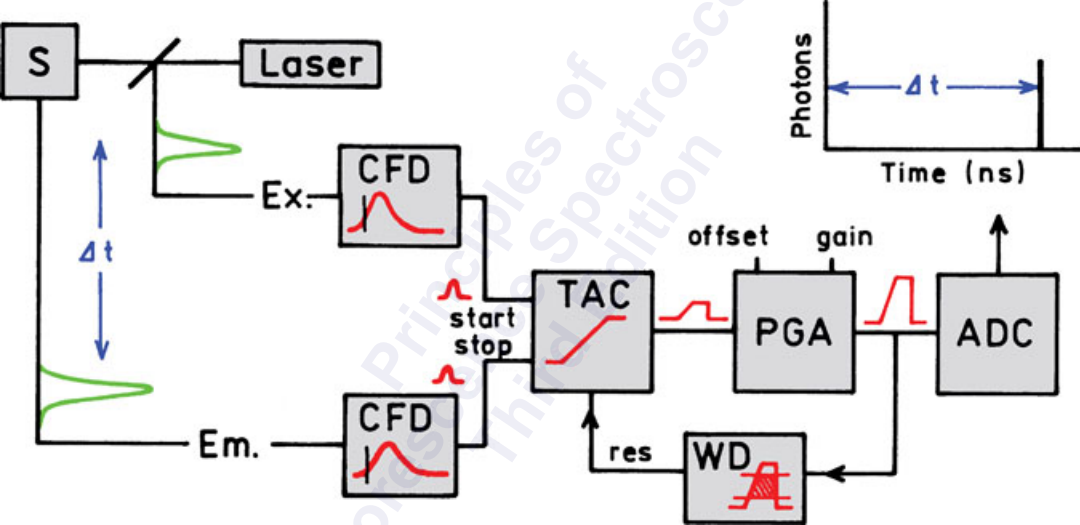


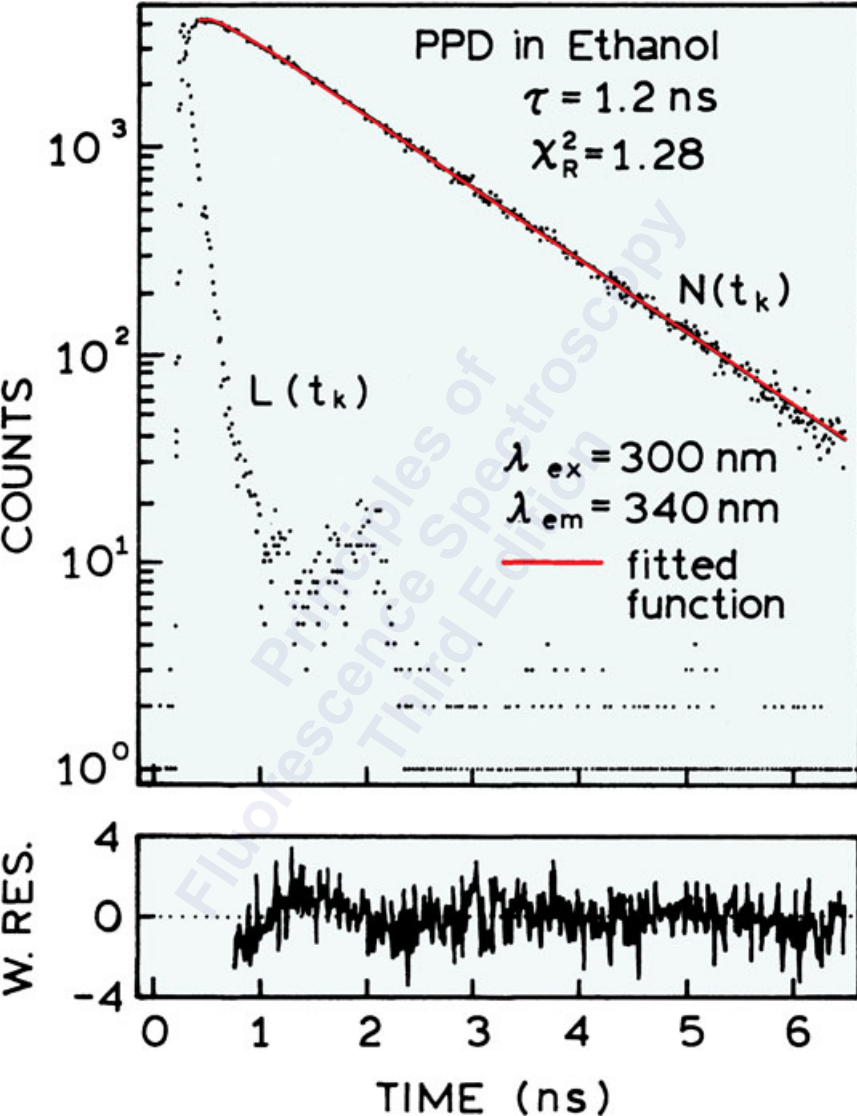
4



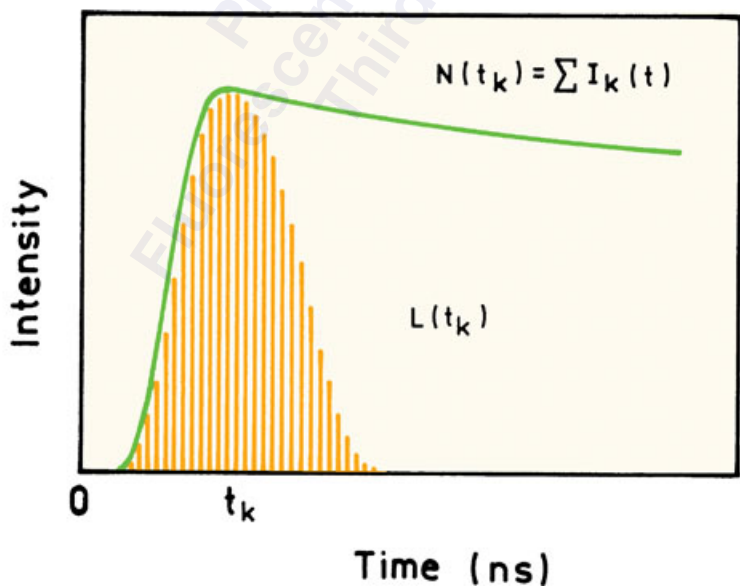
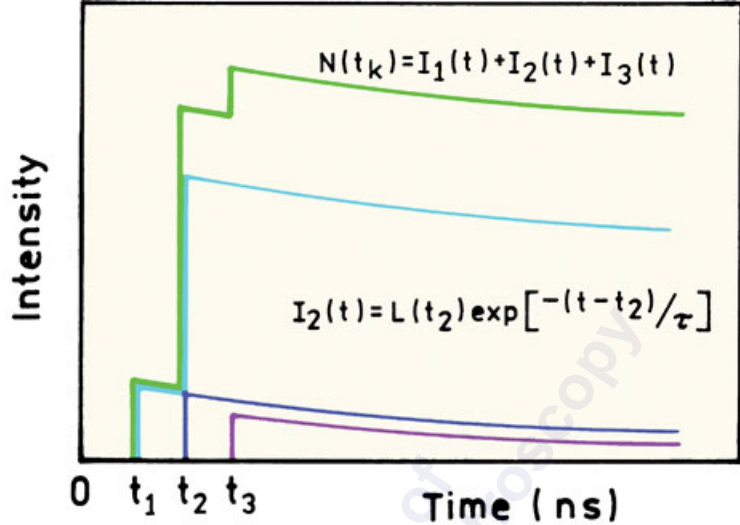




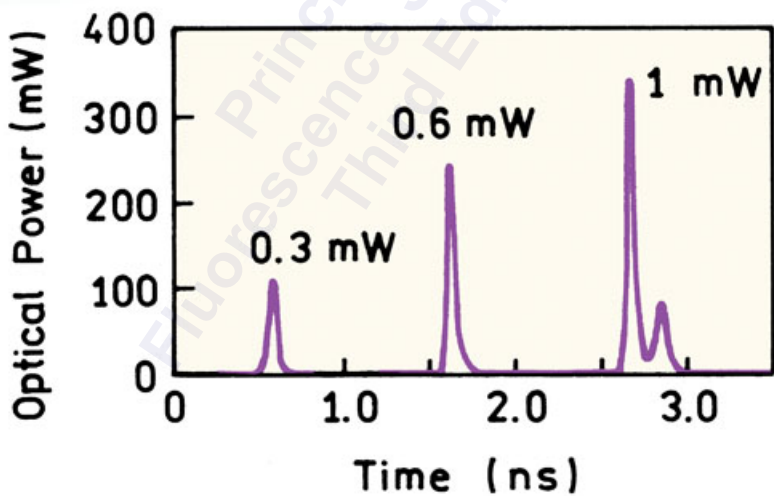


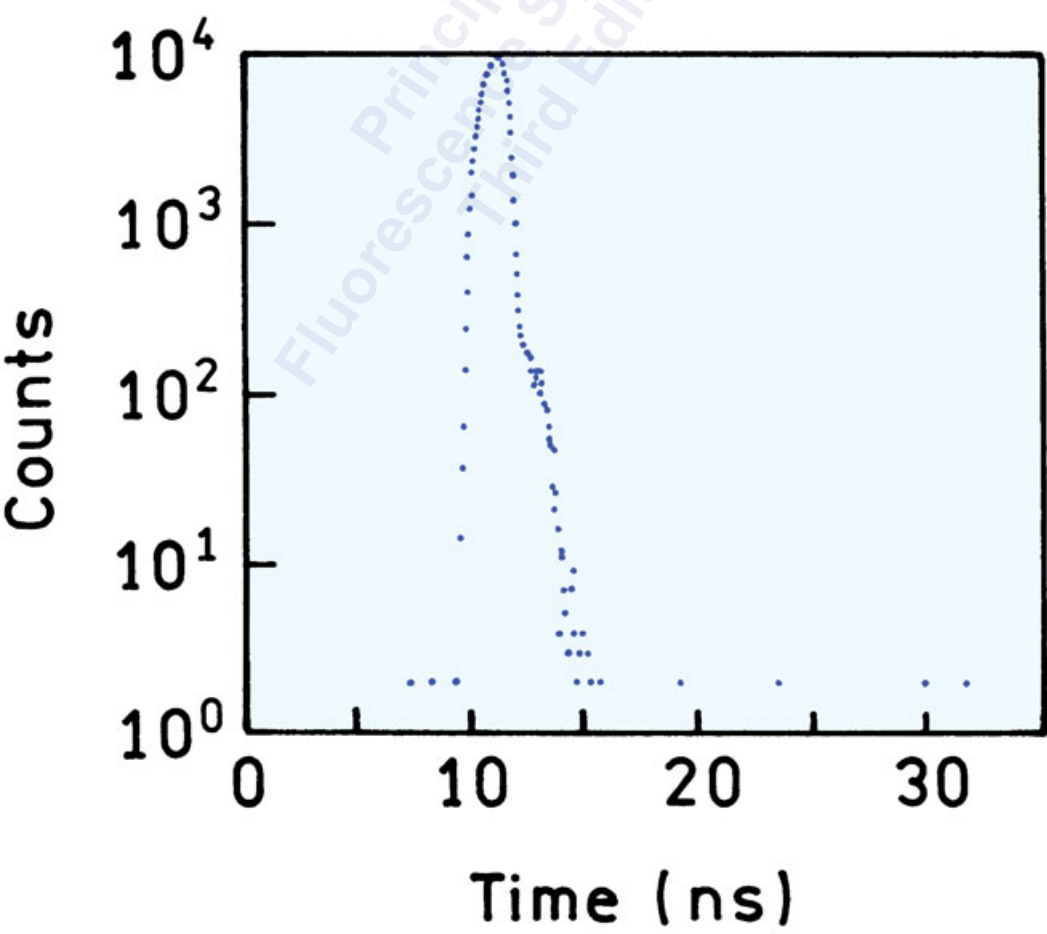




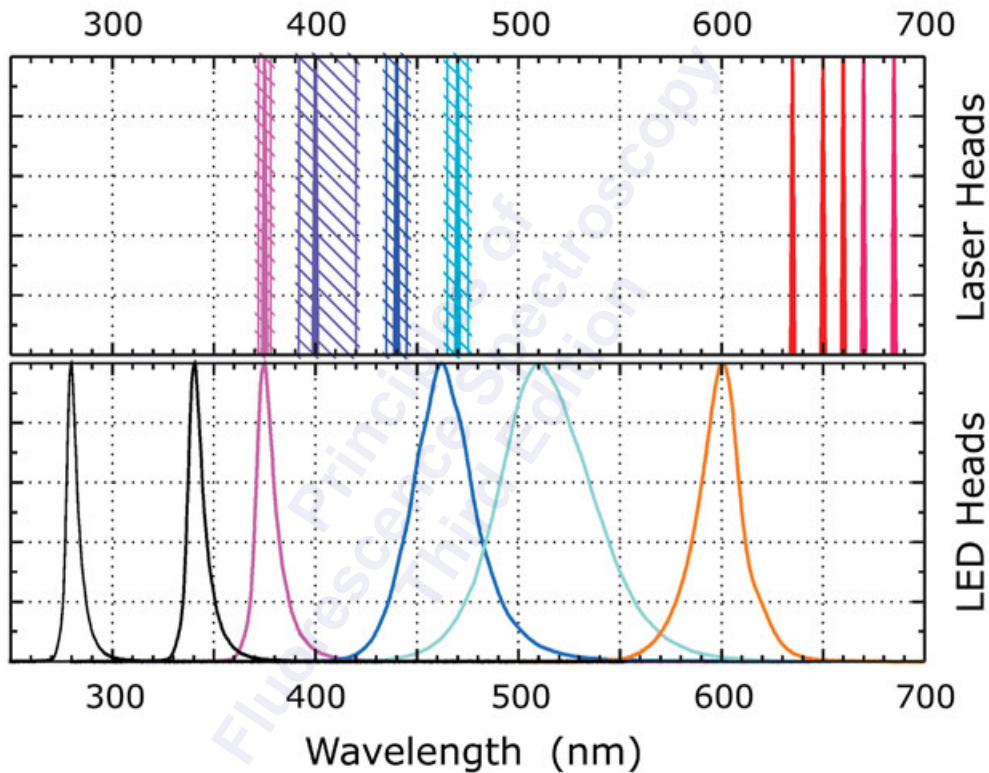


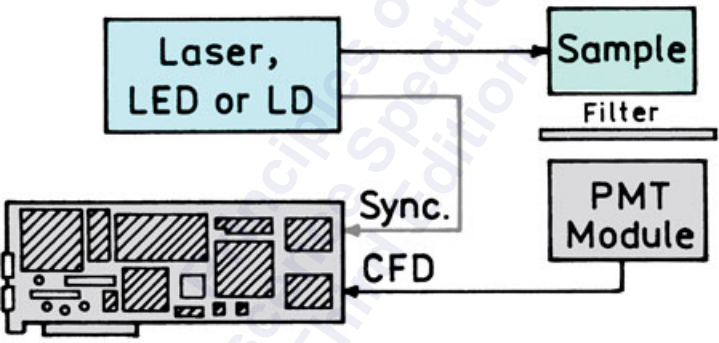


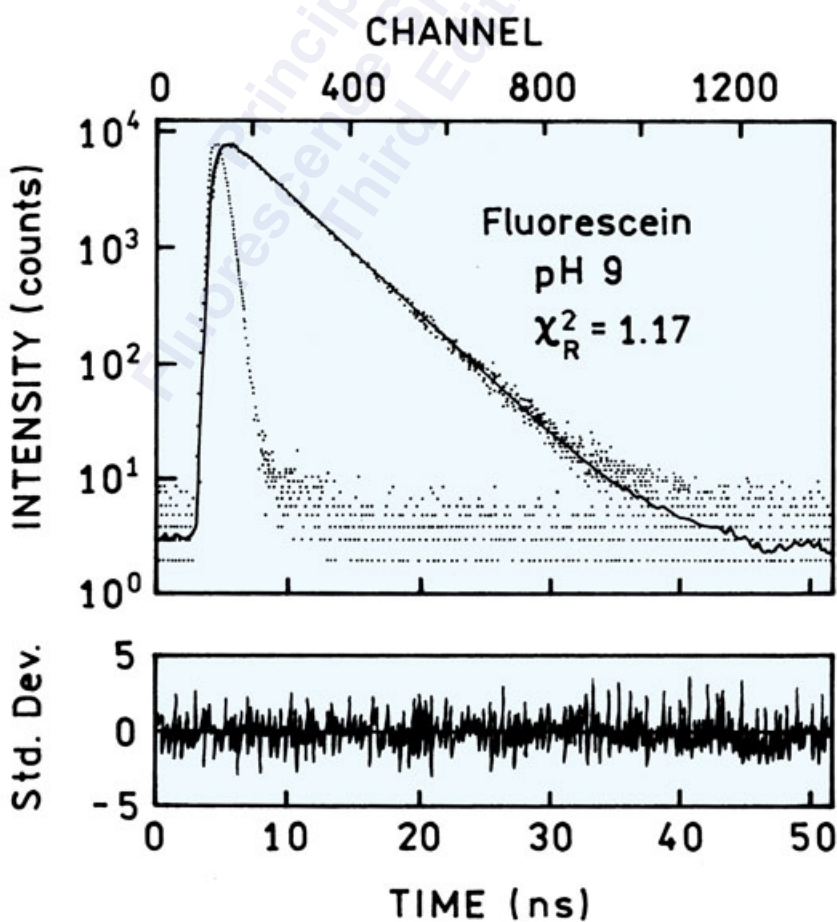
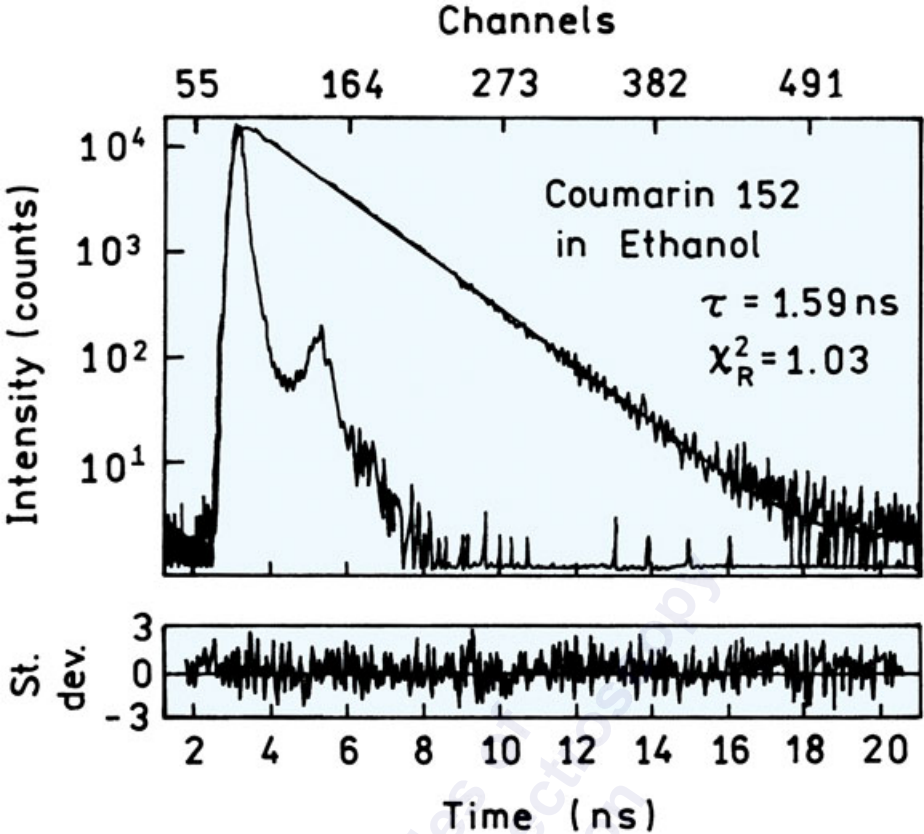


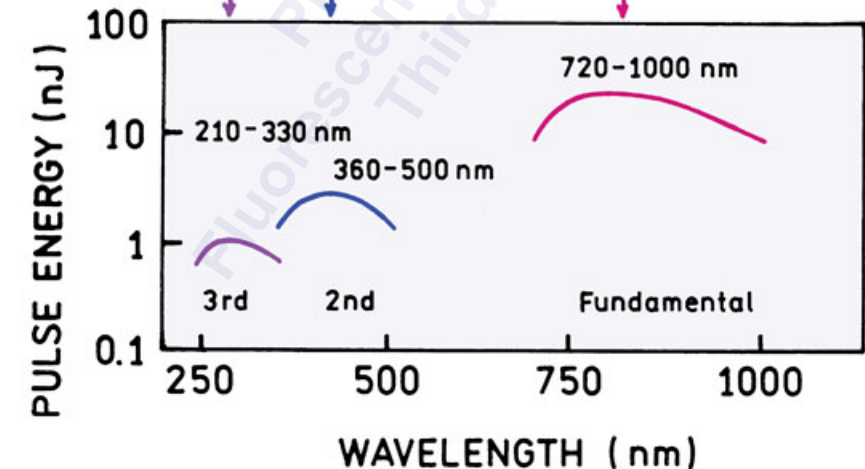
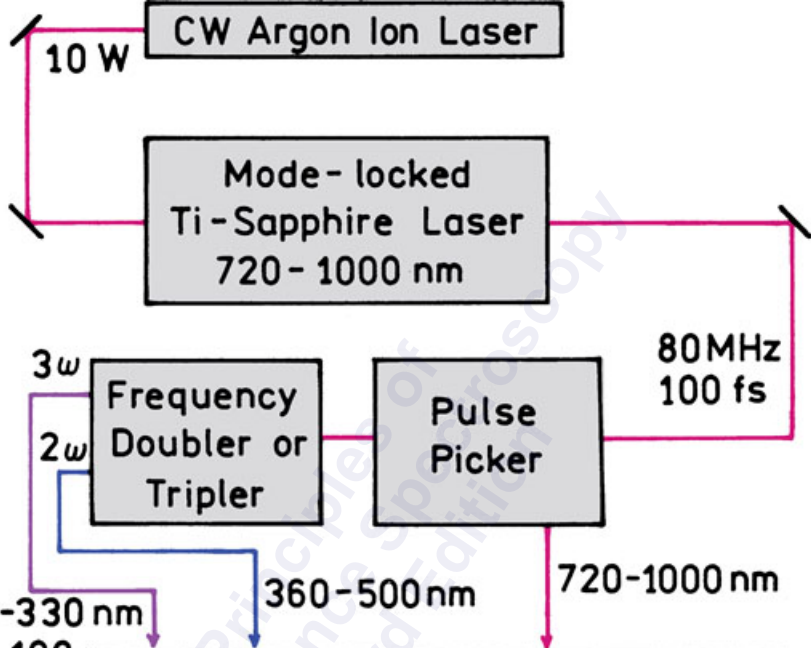


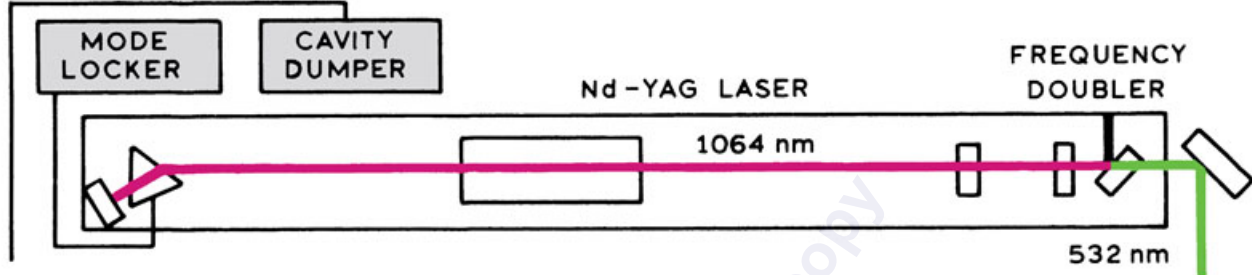
Normalized Emission Intensity



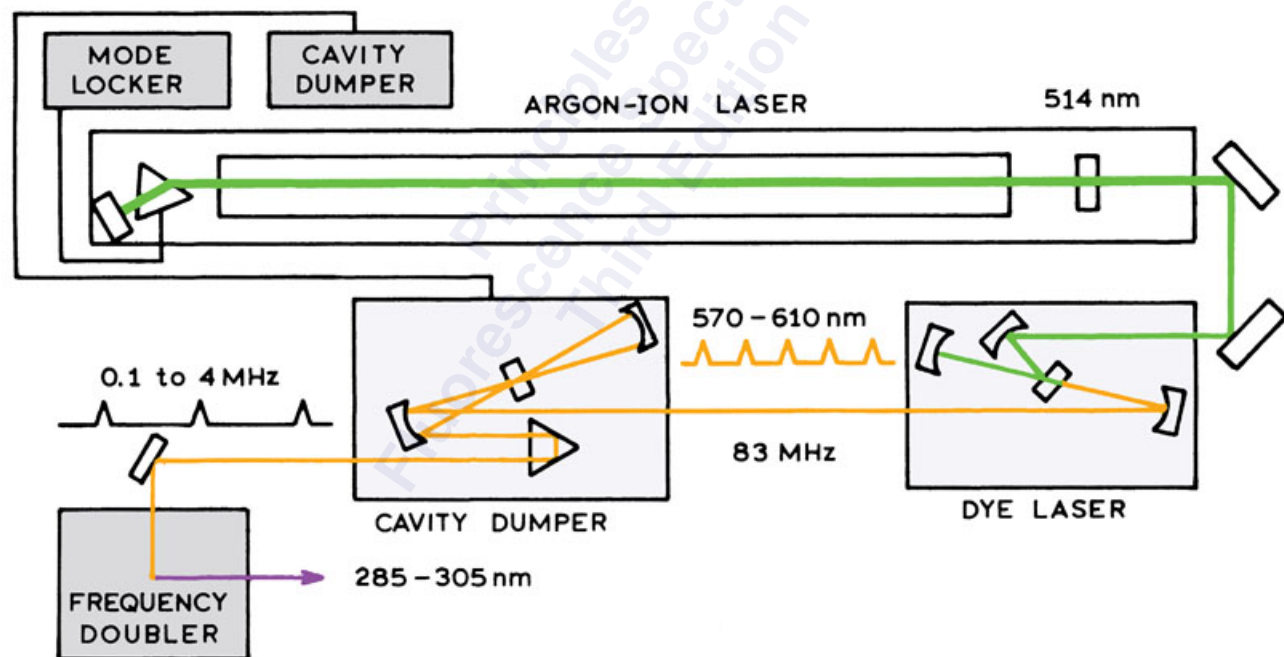






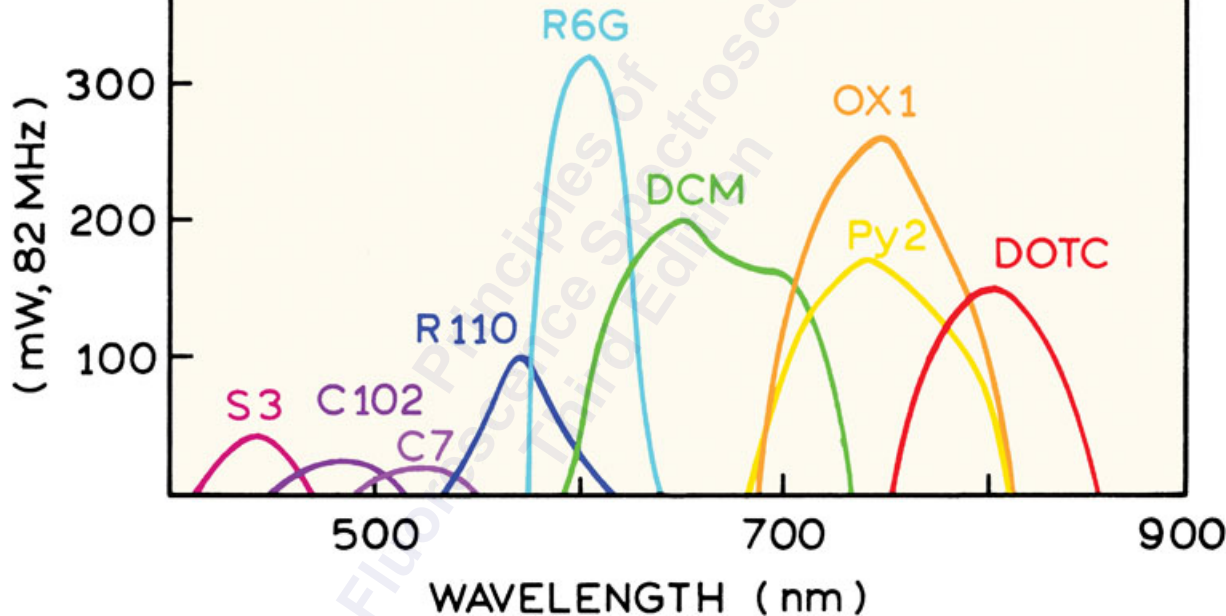


OR

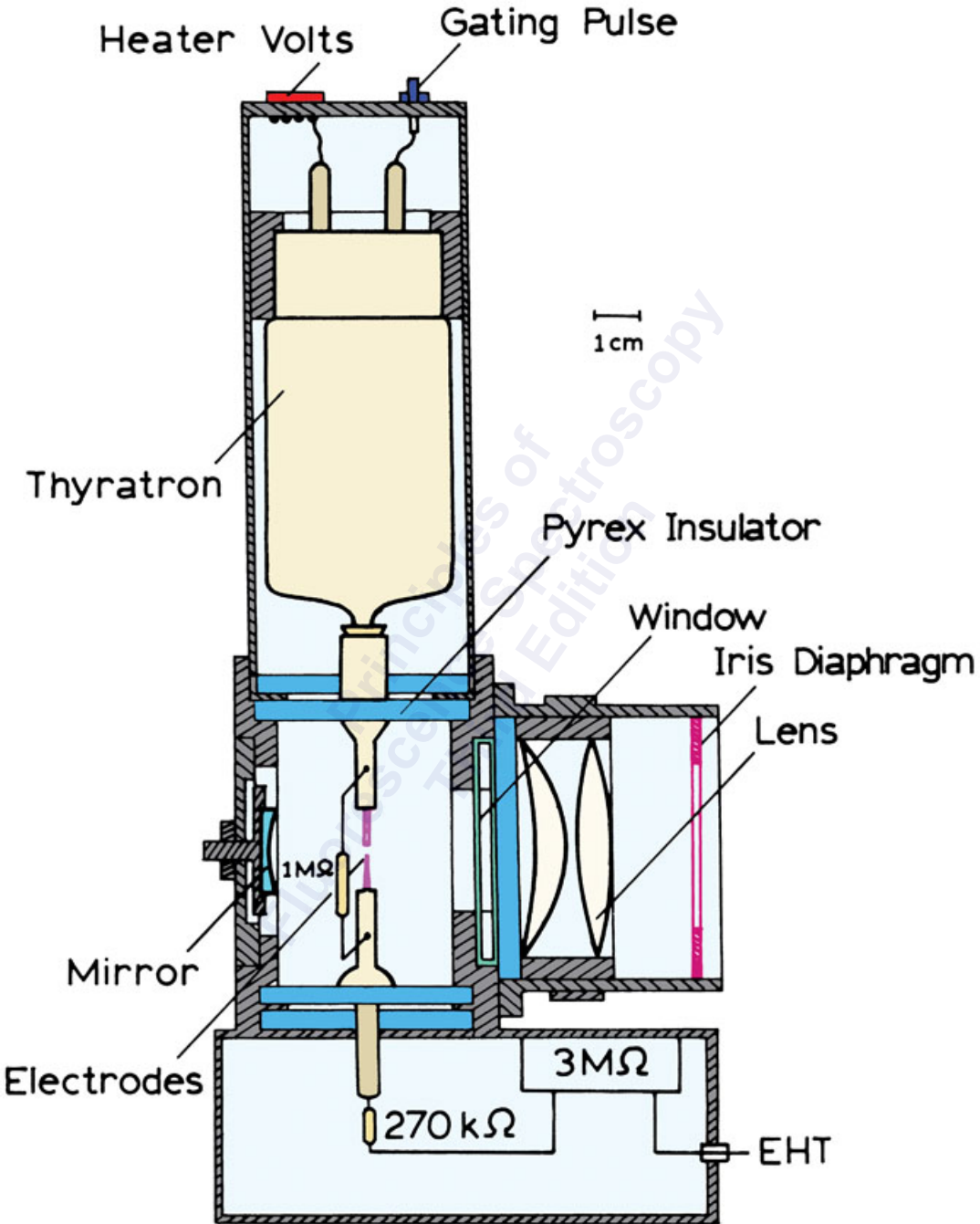


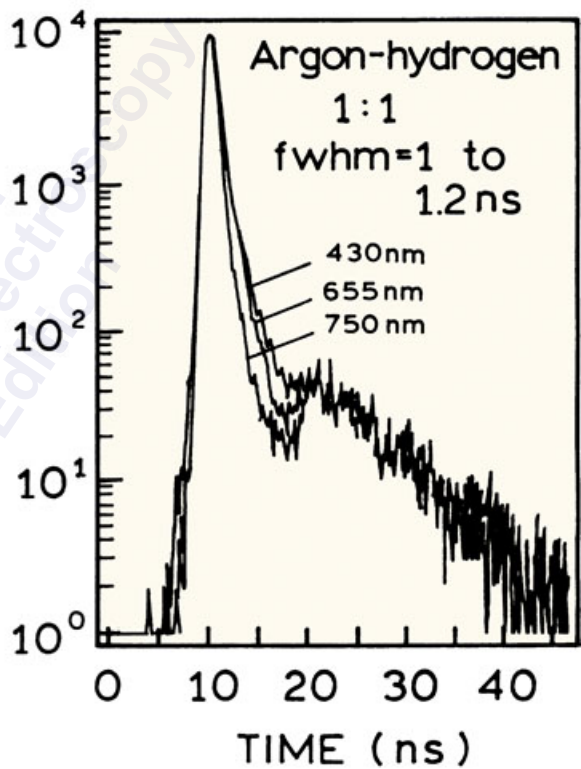
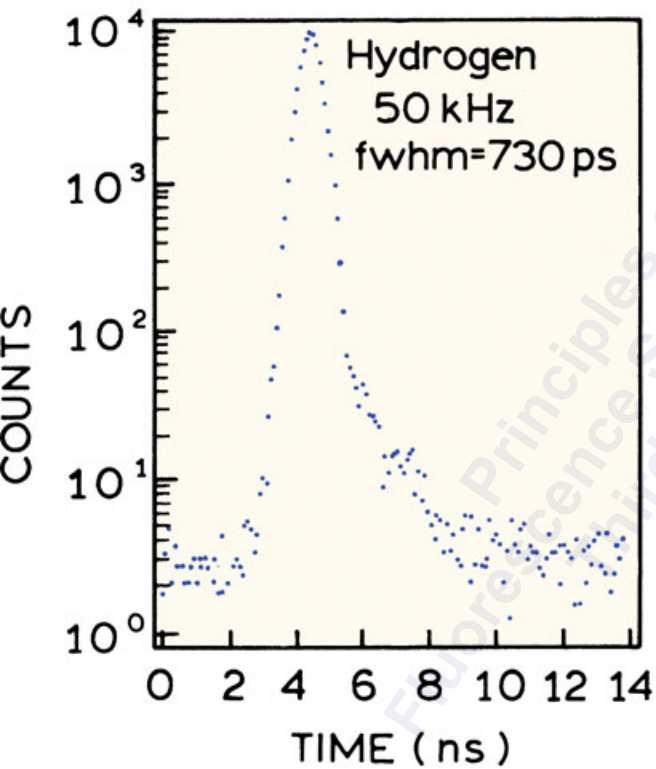


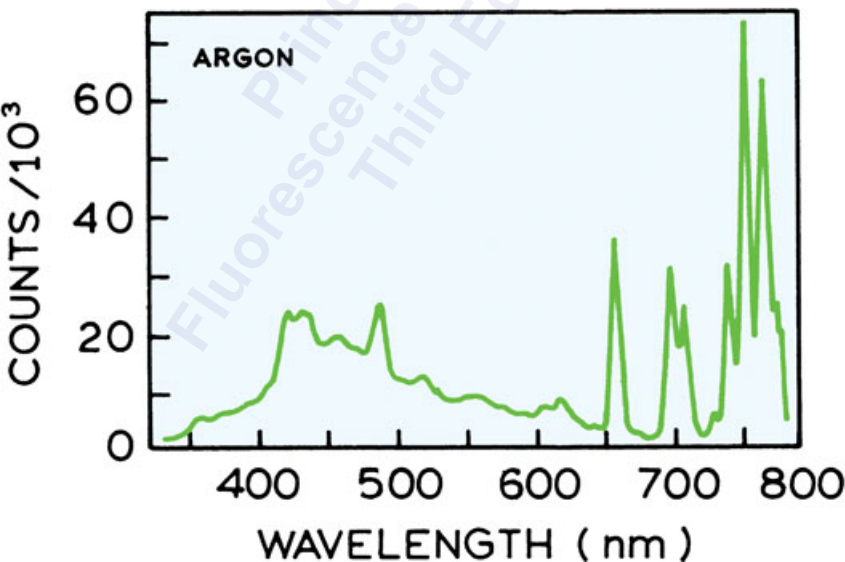
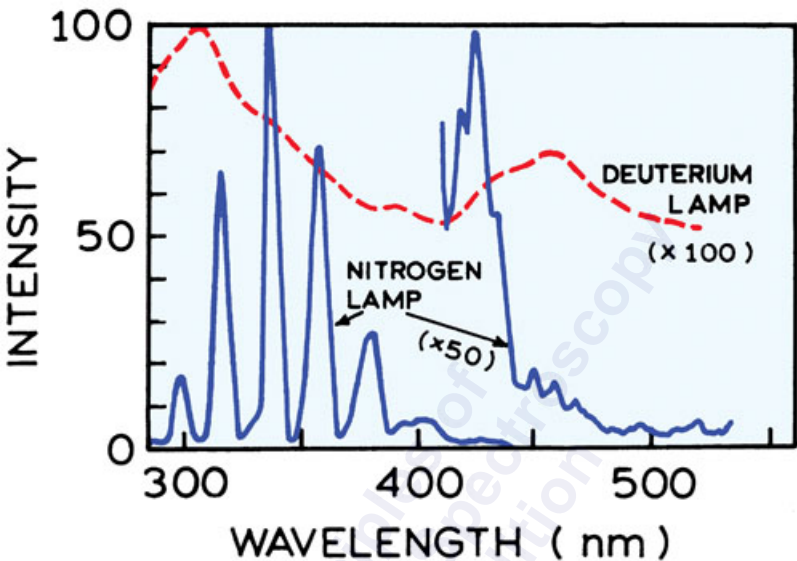
AVERAGE POWER  
(mW, 82 MHz)



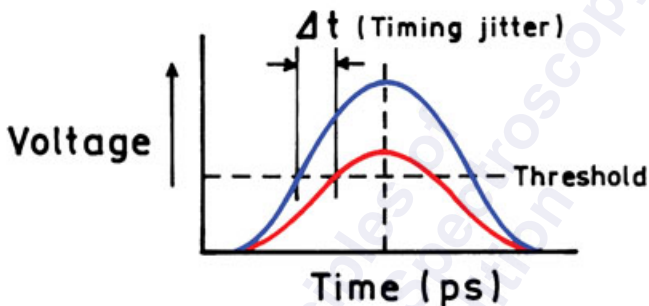




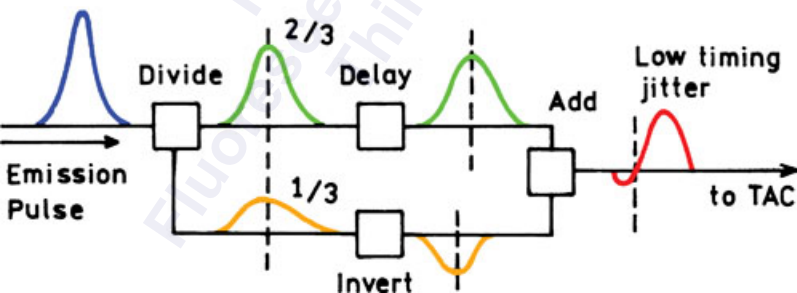


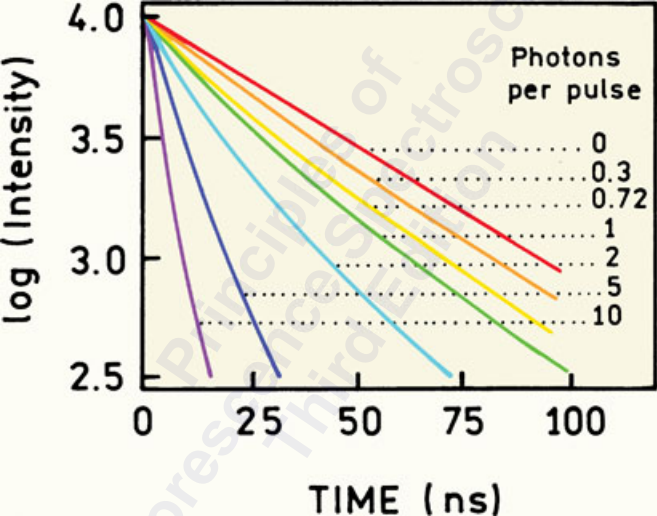


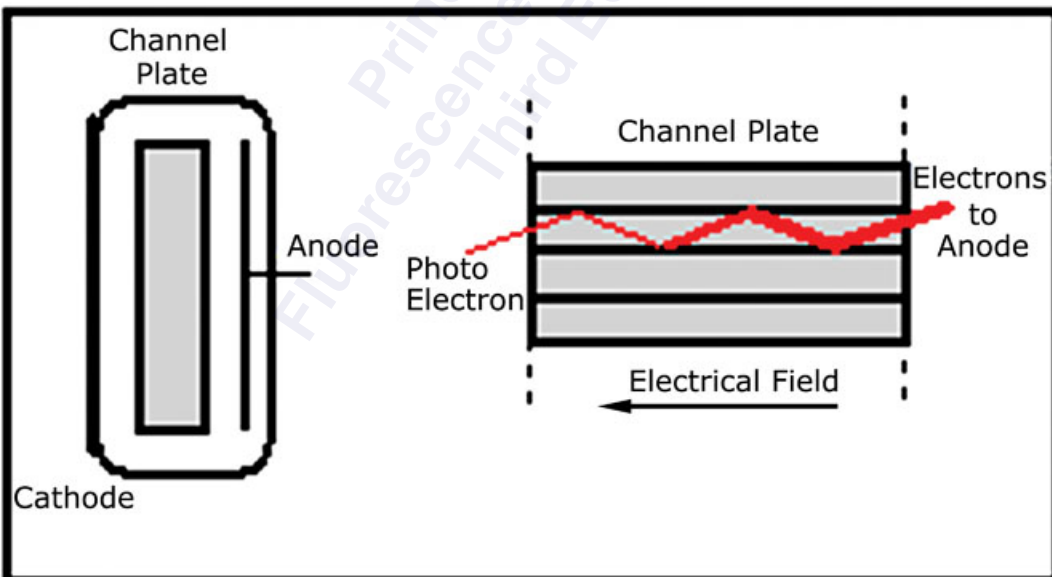
## Leading Edge Discrimination

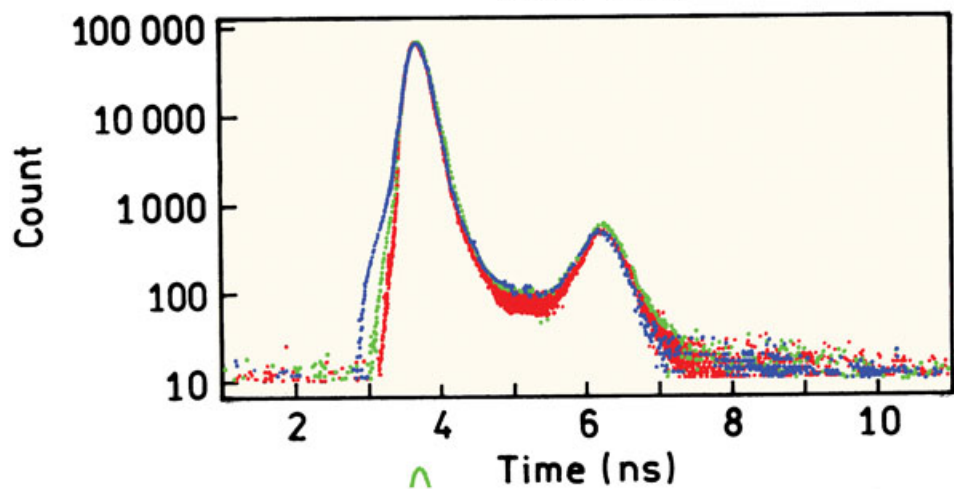
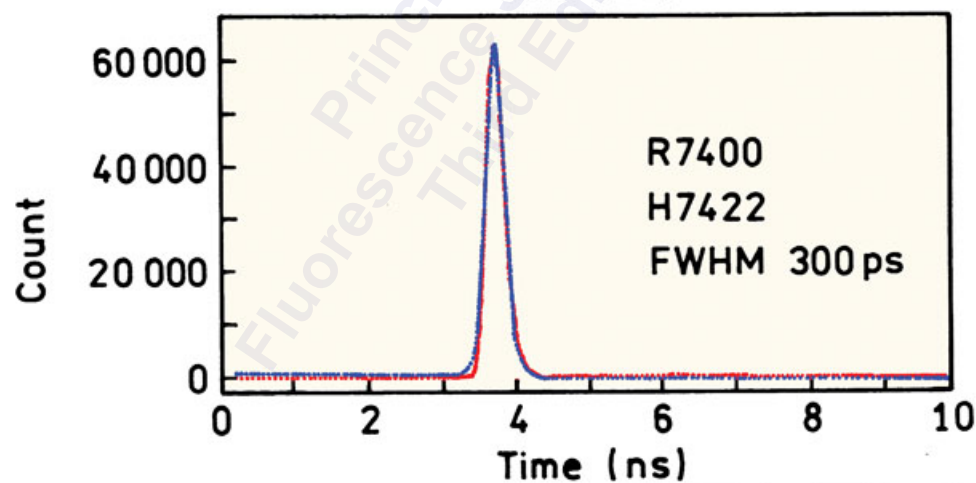
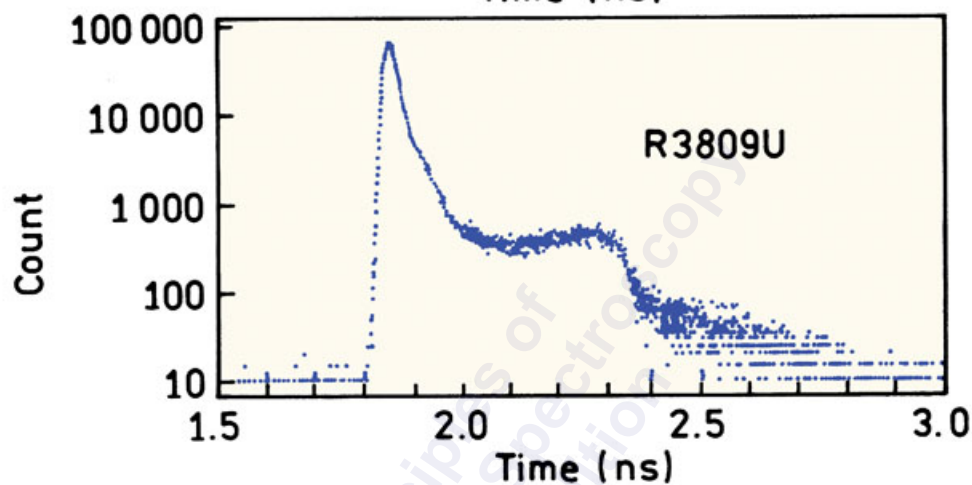
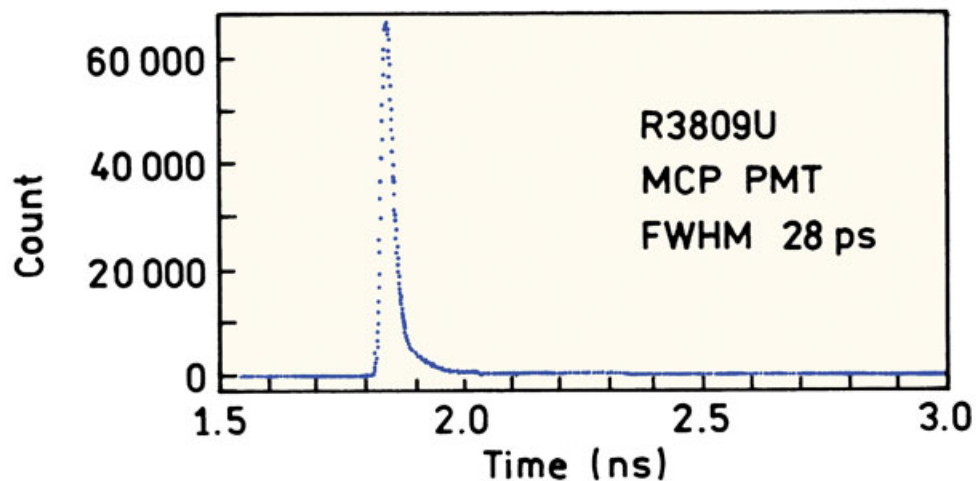


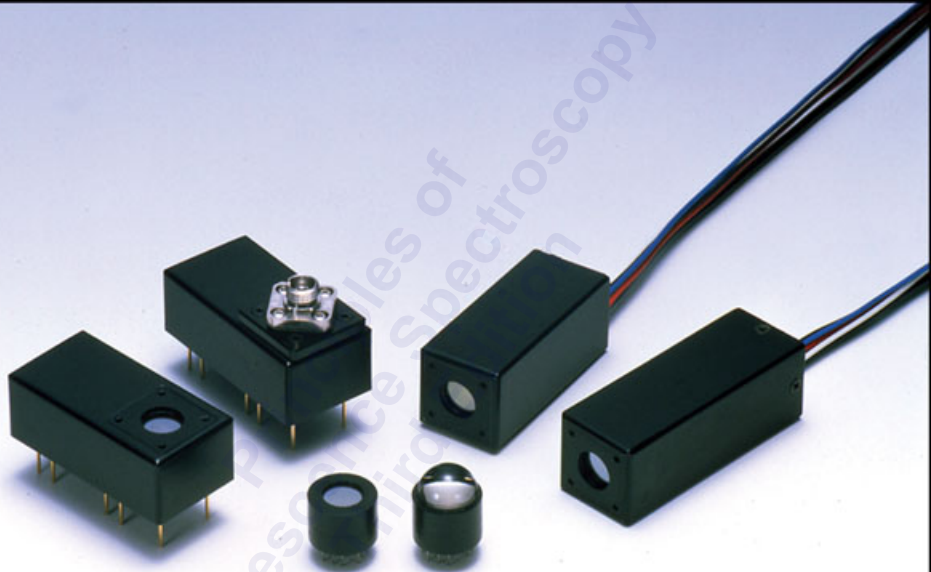
## Constant Fraction Discrimination



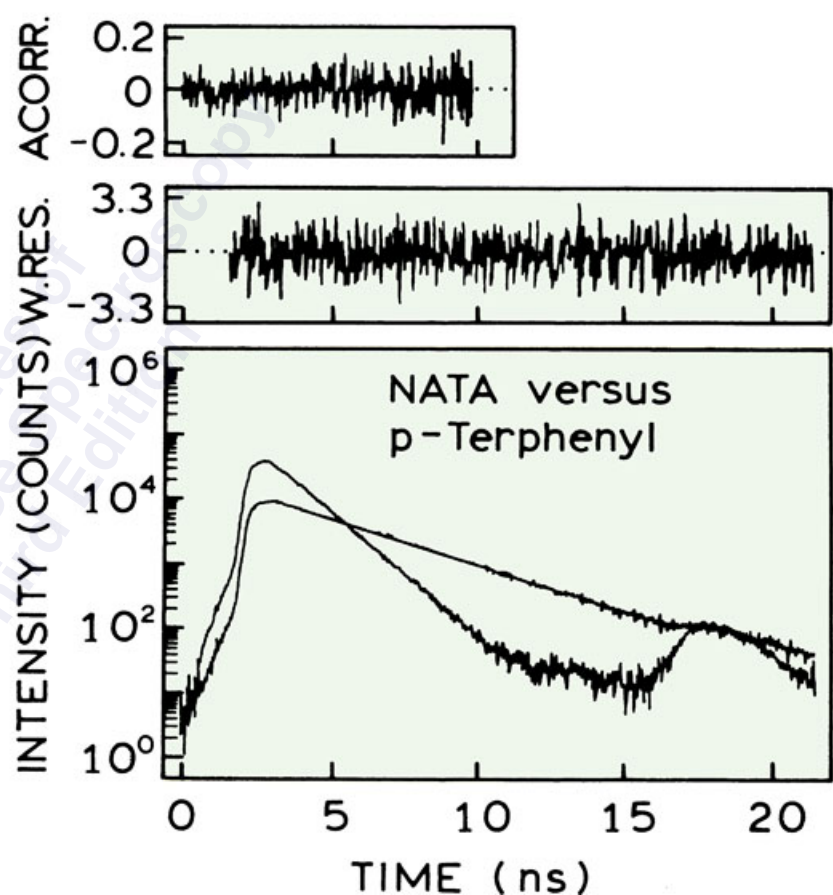
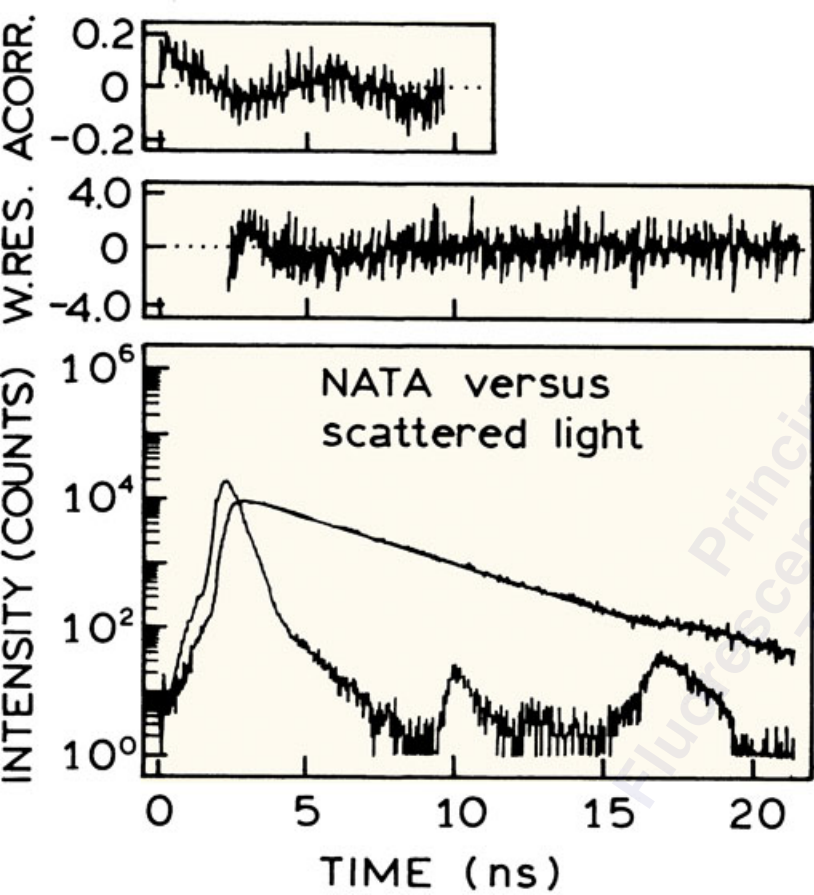


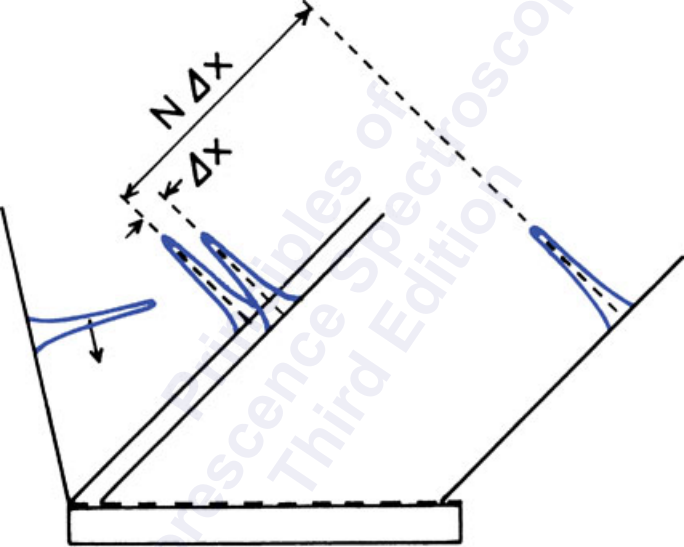


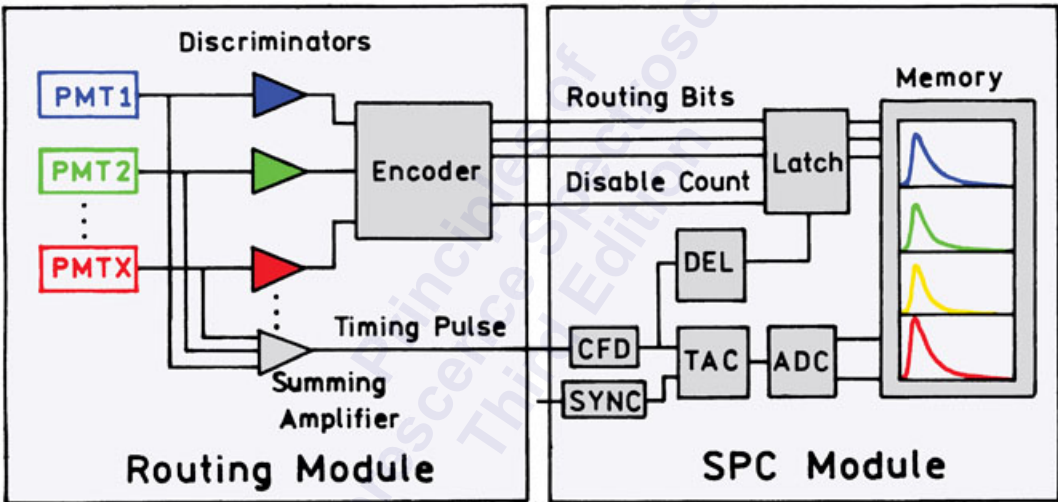




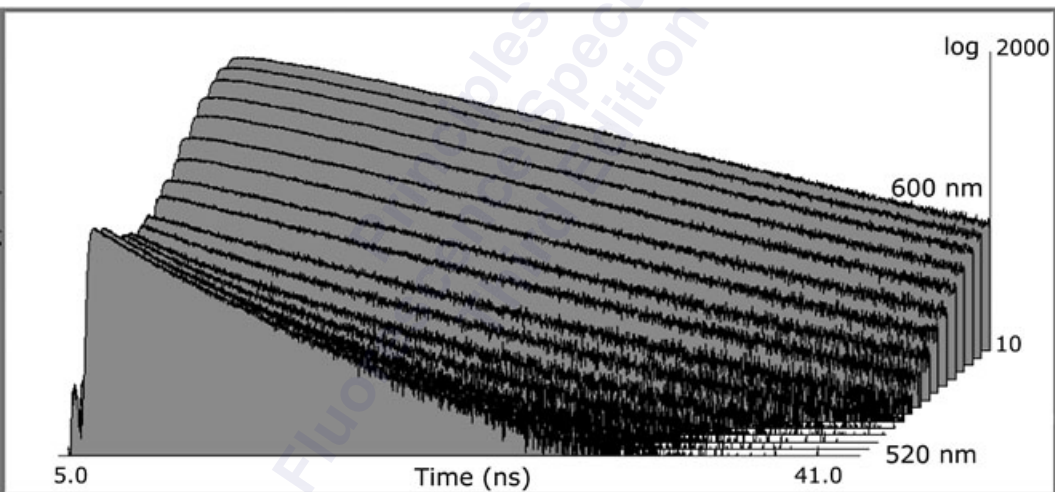
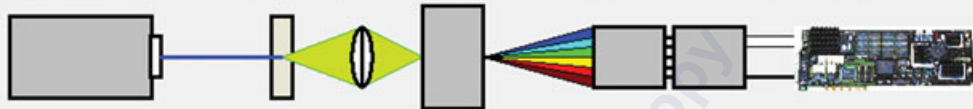


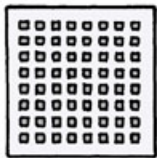






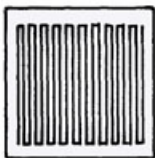
ps Diode Laser      Rhodamine 6G      Polychromator      16 Ch. PMT      Routing      Multi-Dimensional  
 405nm, 50ps, 20MHz      + Fluorescein      MS 125-8M      R5900-L-16      electronics      TCSPC Module





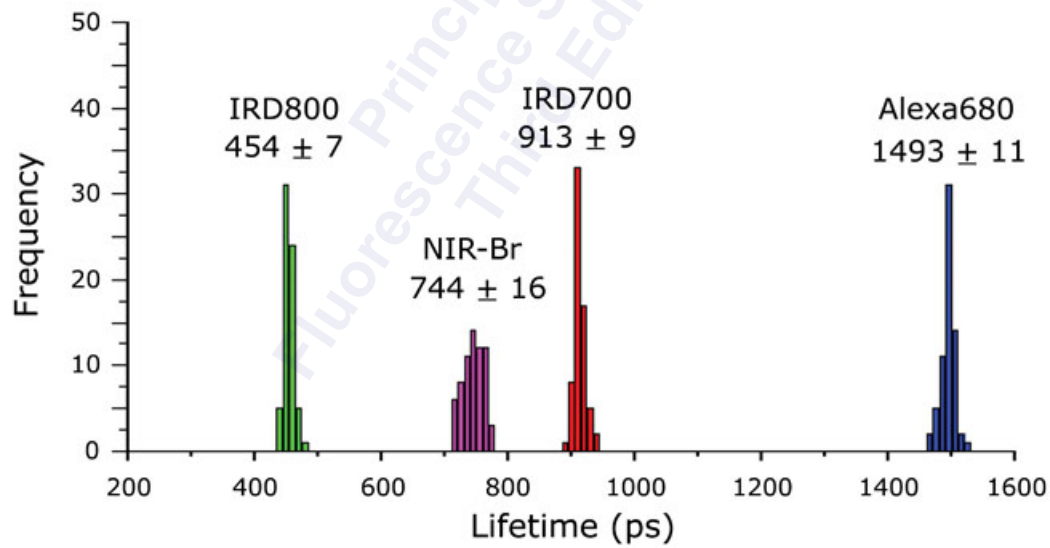
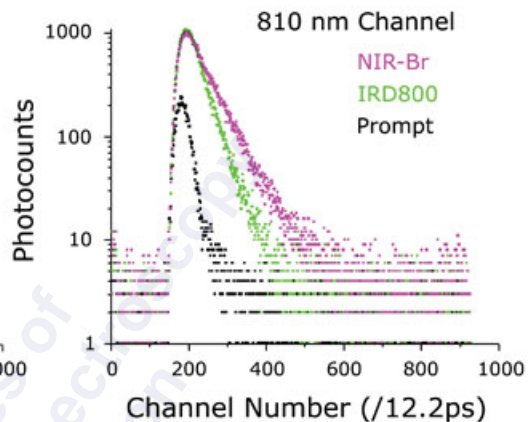
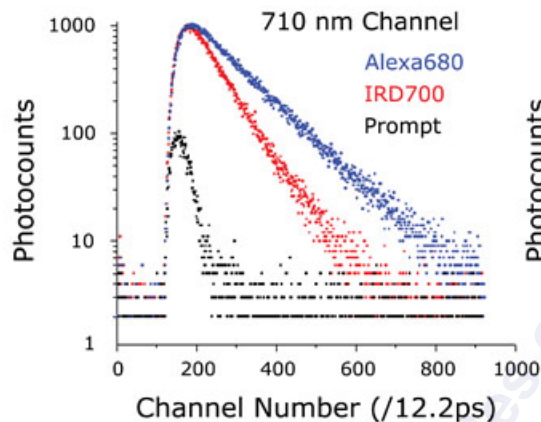
$16 \times 16$

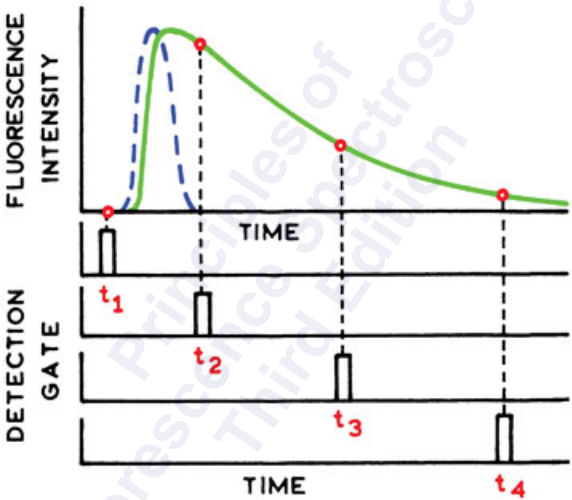
Anodes

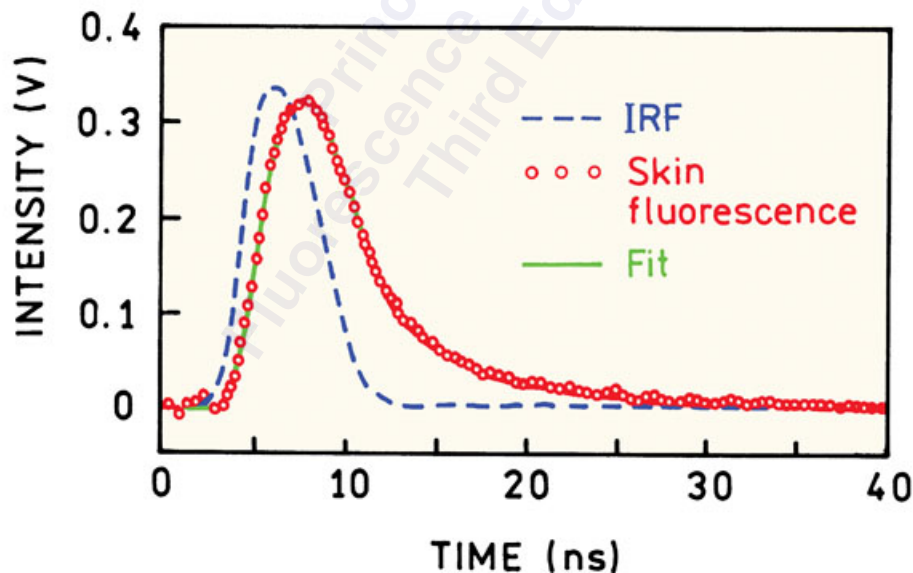
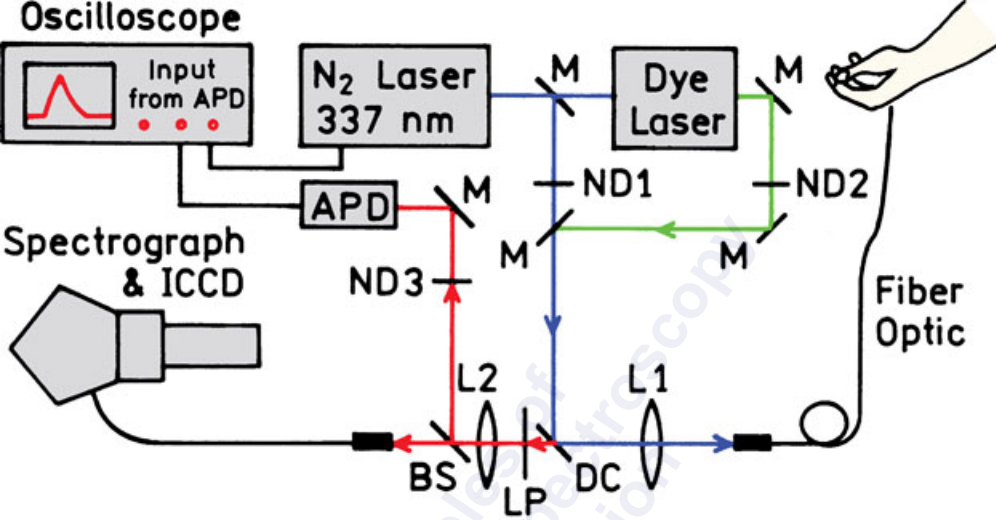


16

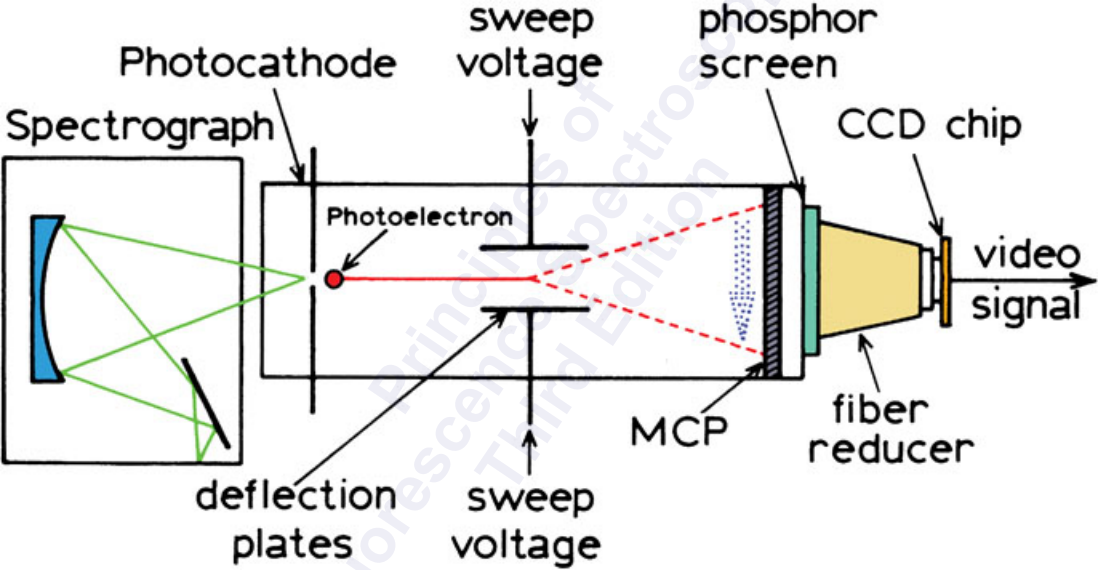


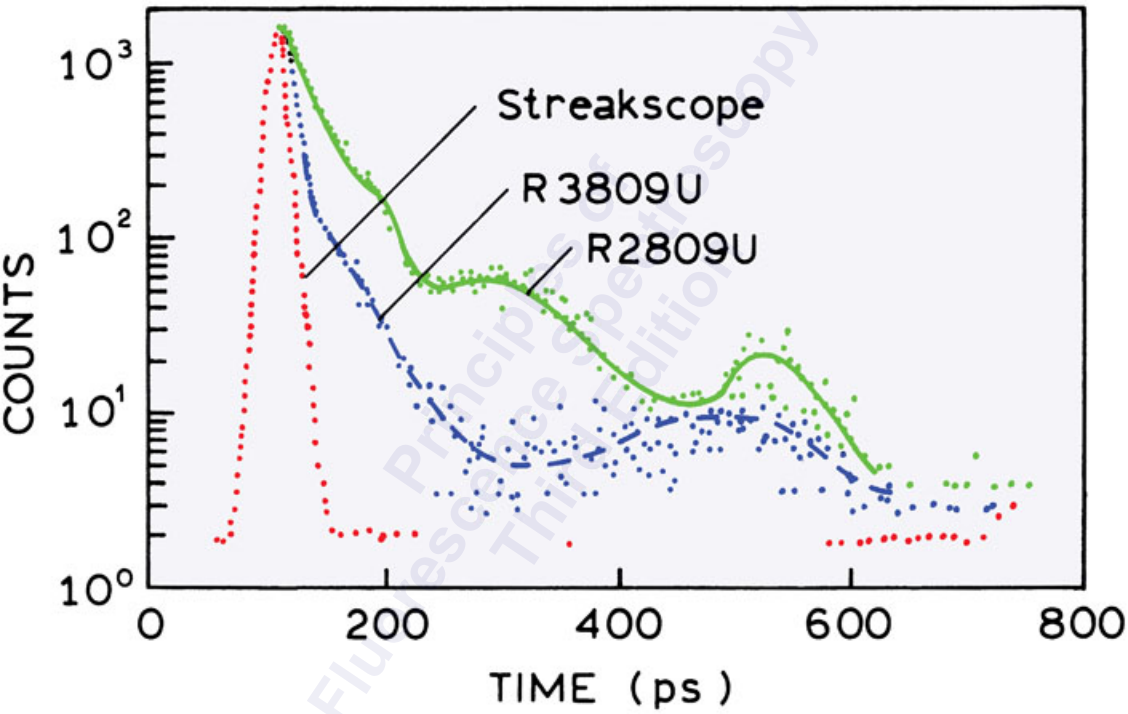


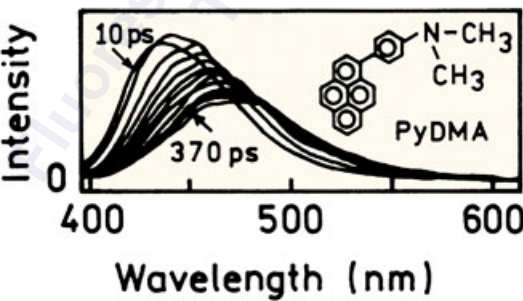
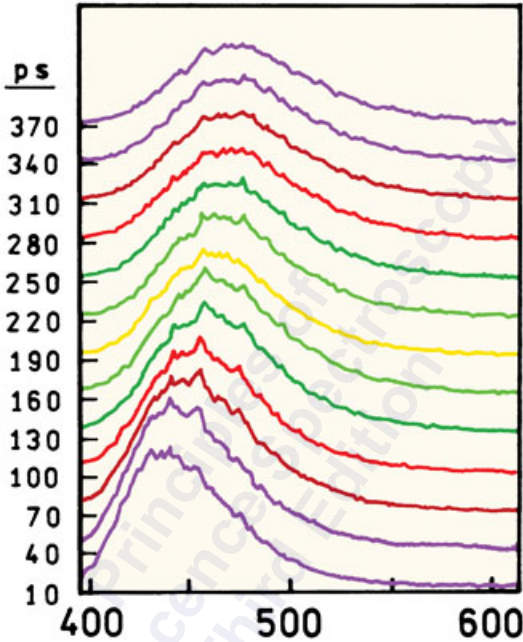


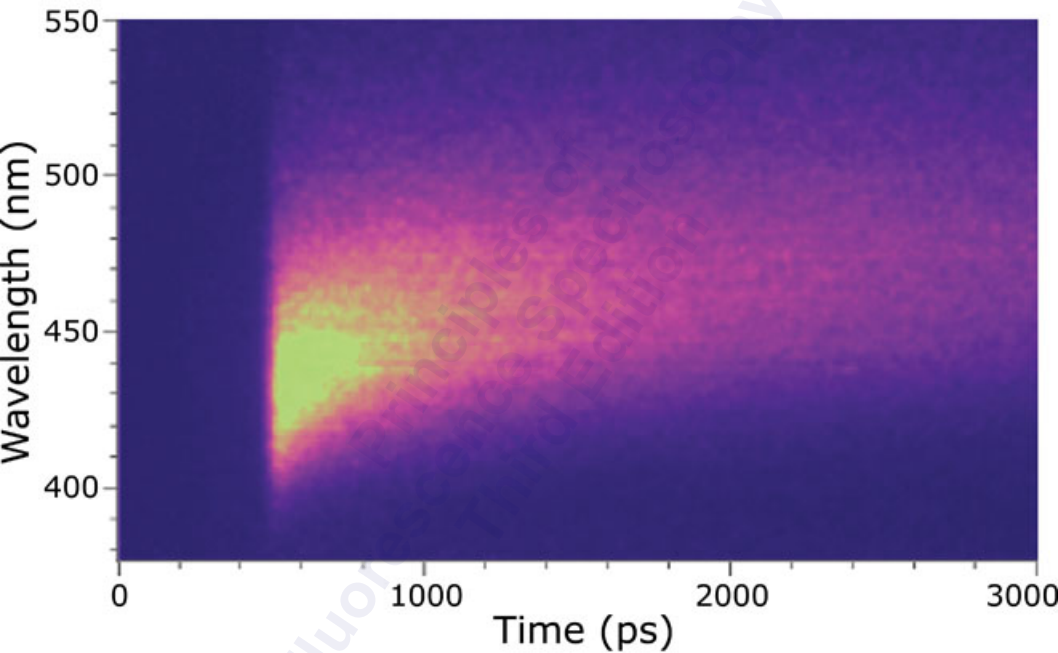


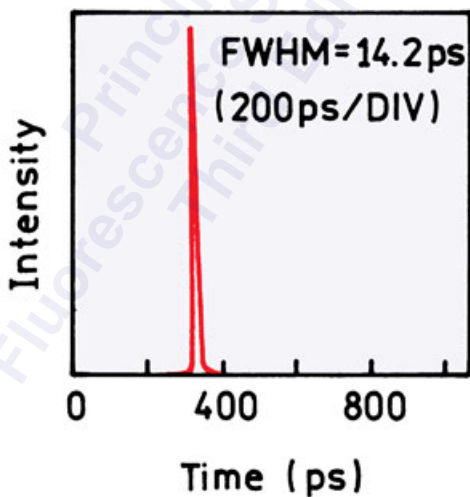
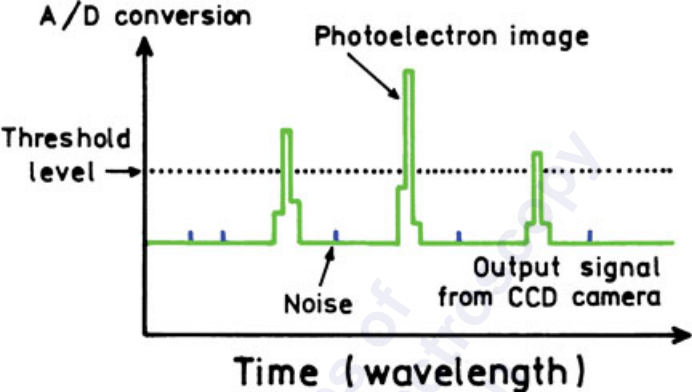






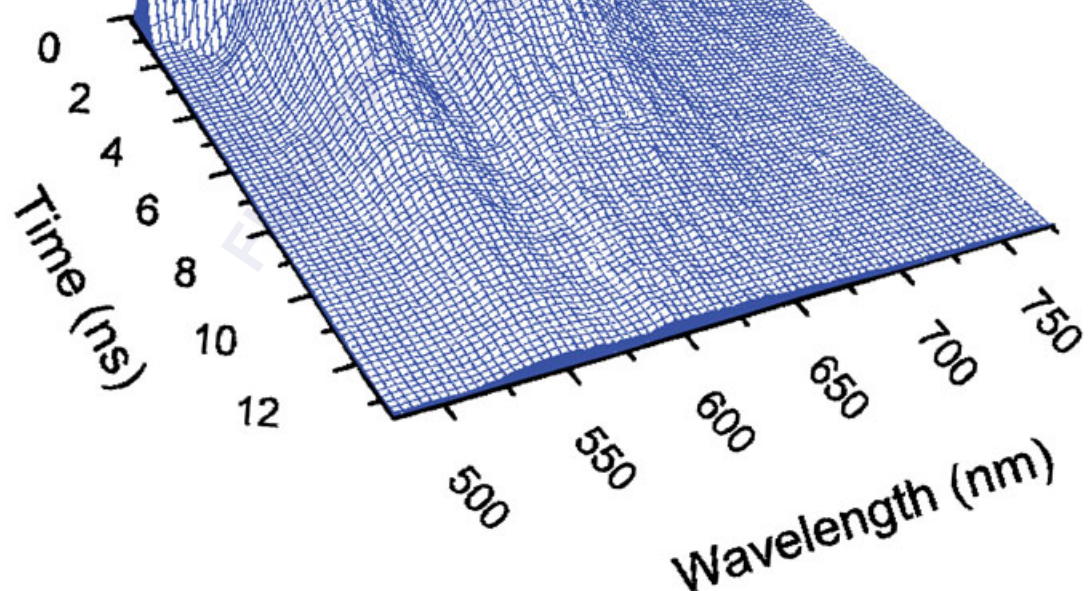


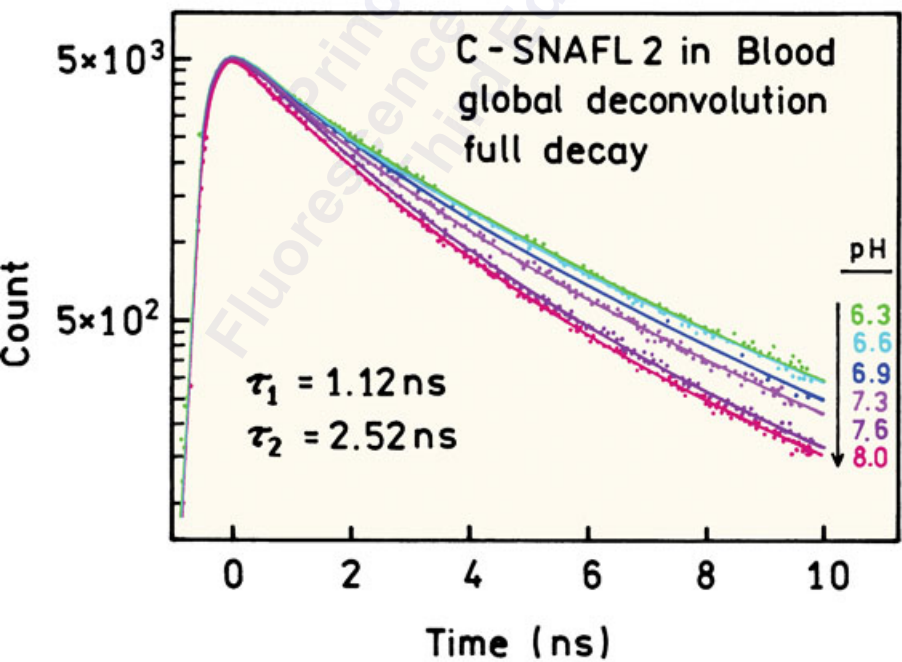
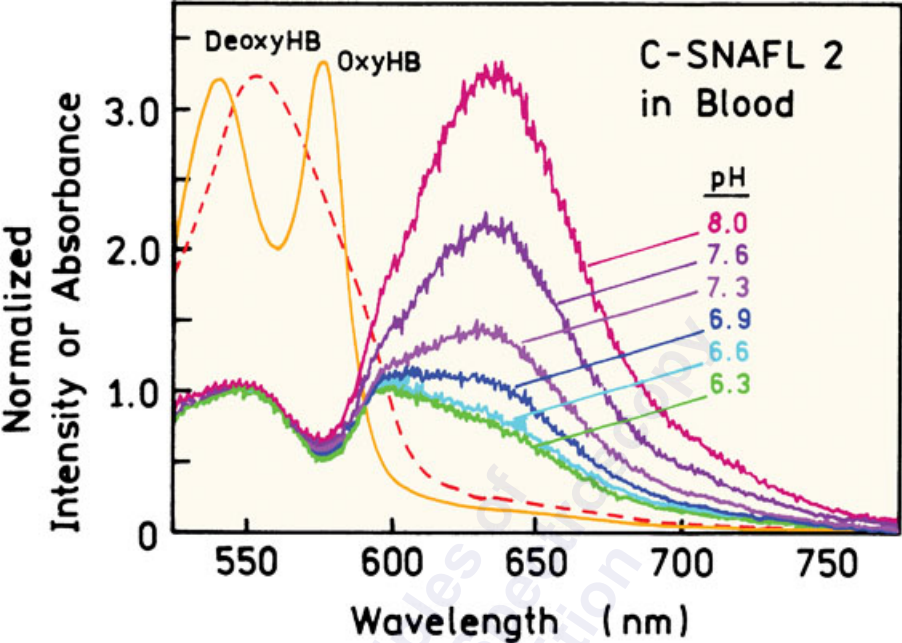




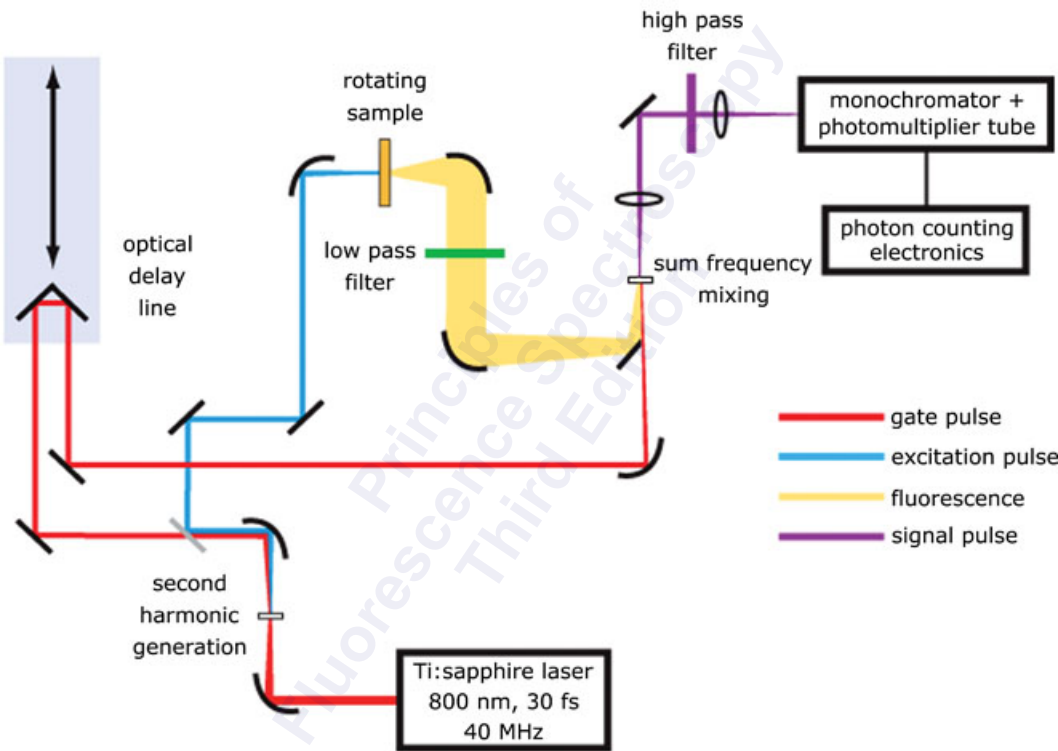
C-SNAFL2 in Blood  
pH 7.3

Intensity

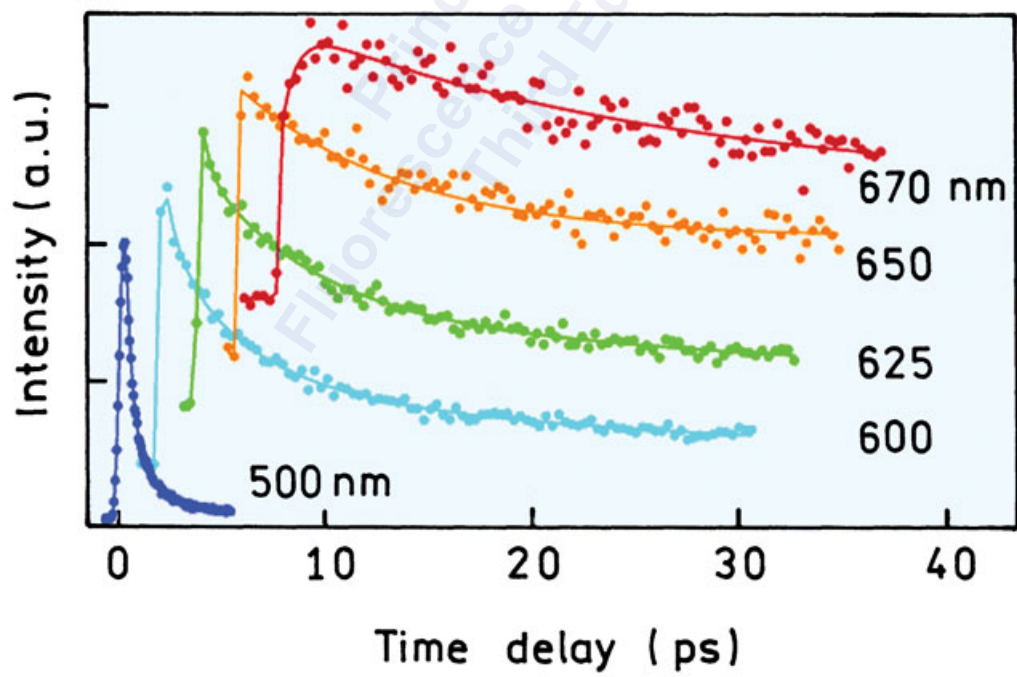
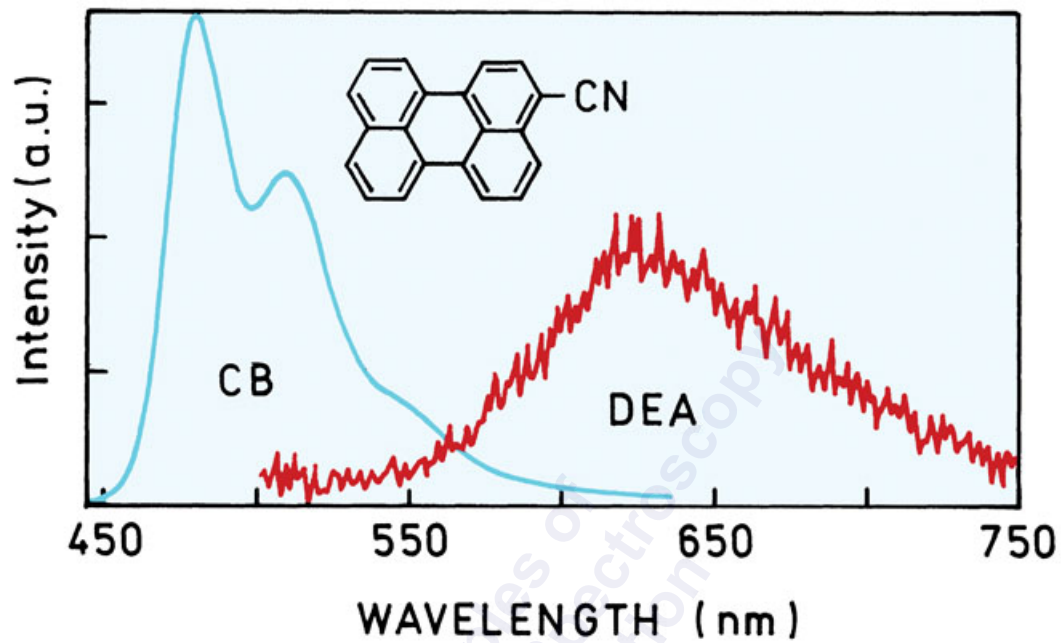




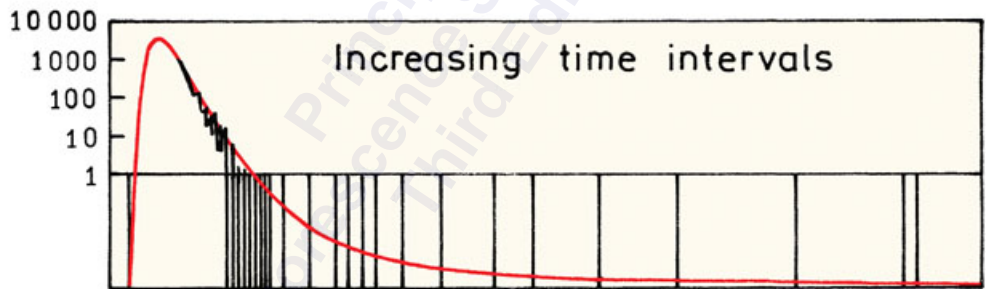
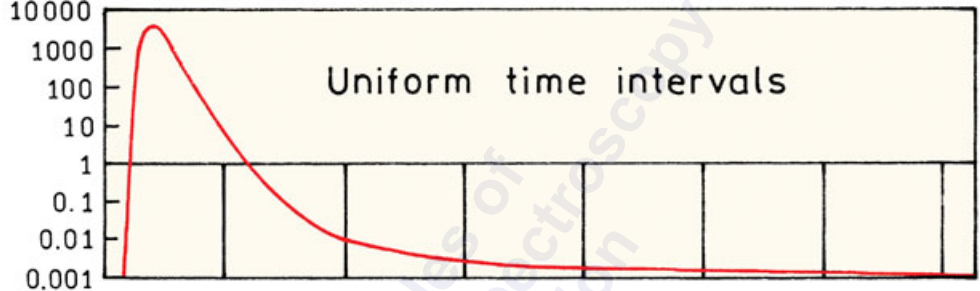




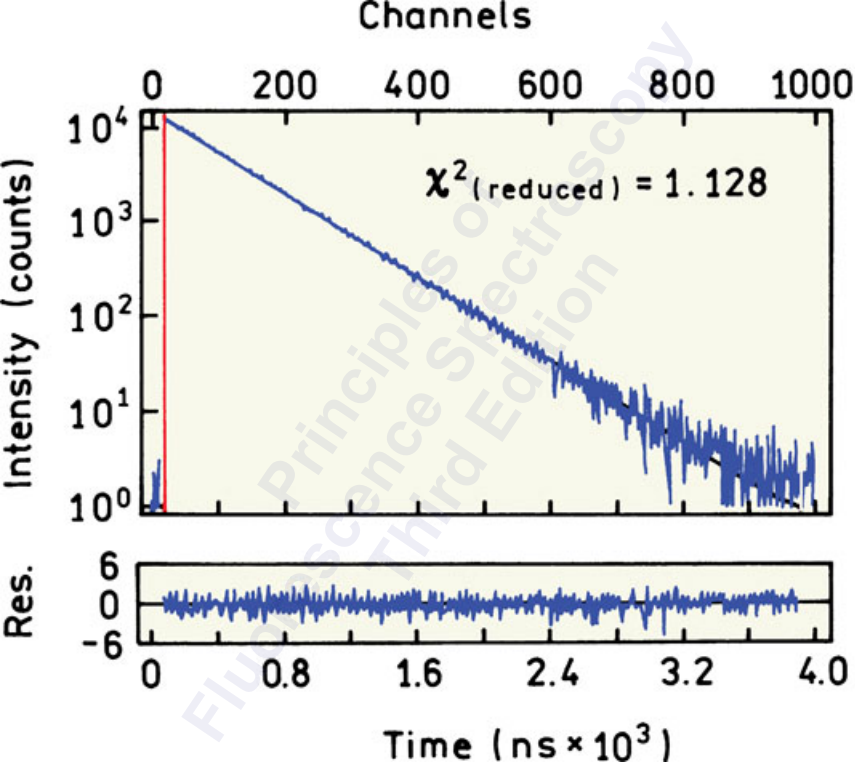




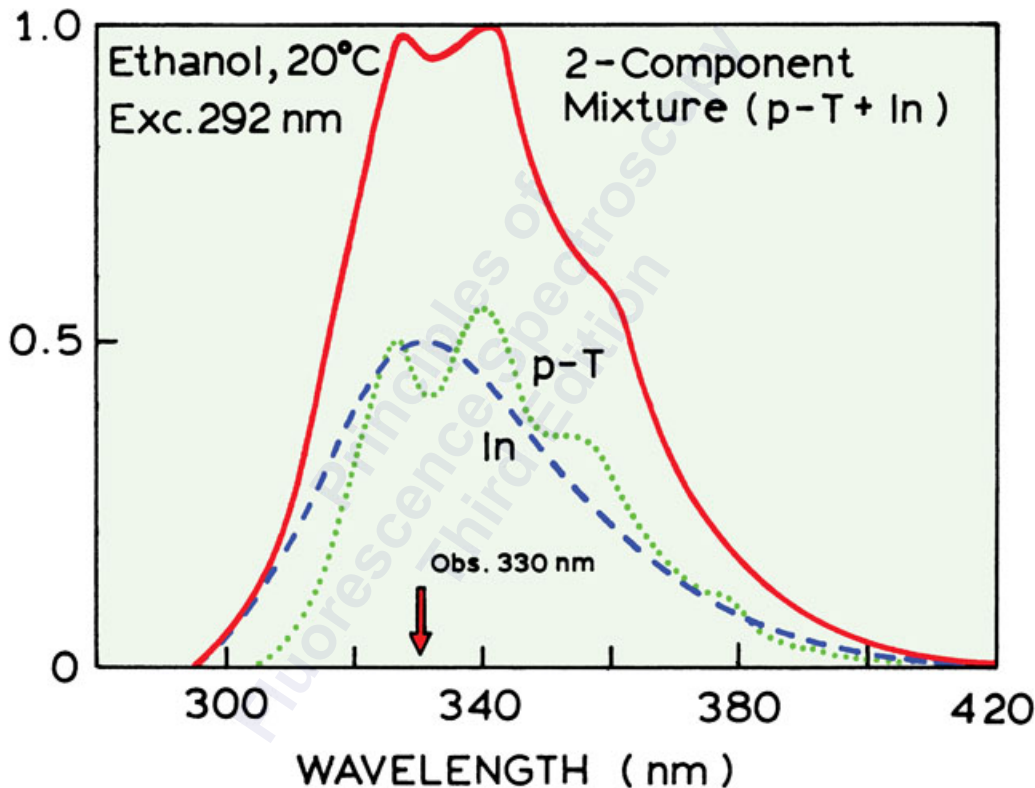
Photons per Time Interval

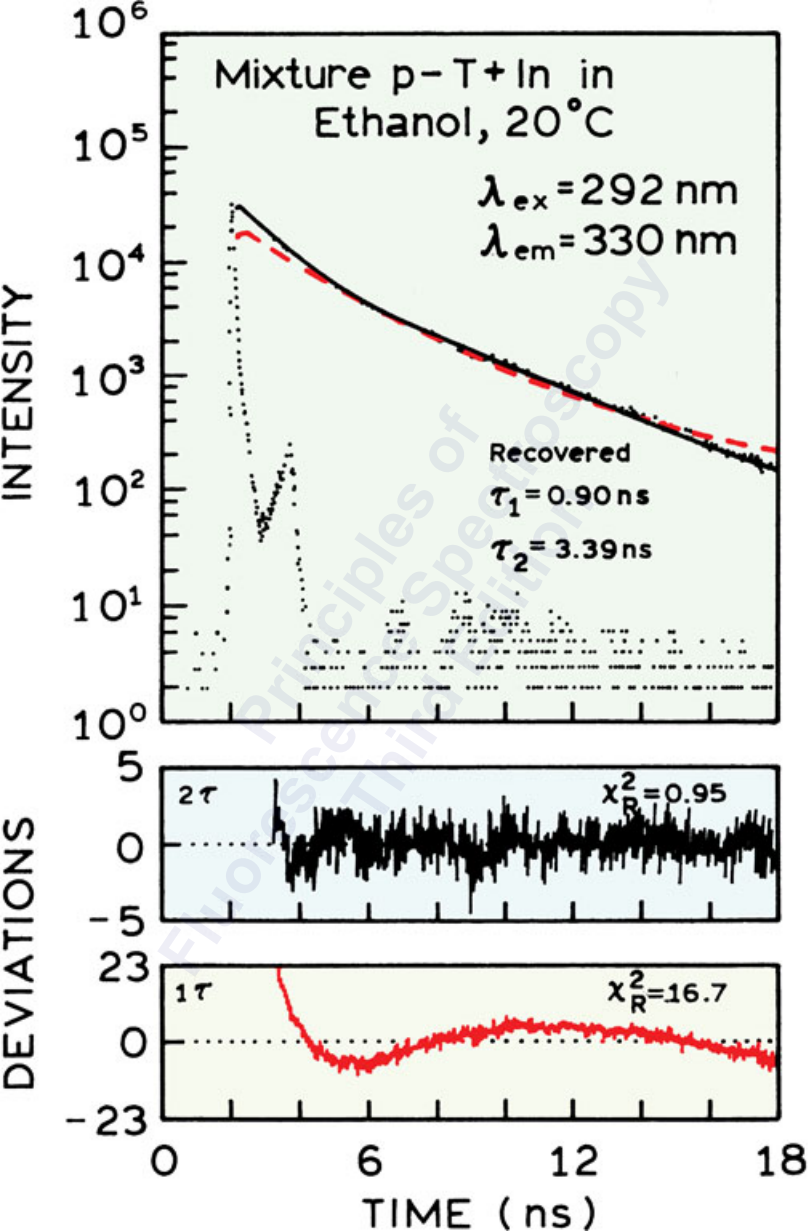


Time ( $\mu$ s to ms)

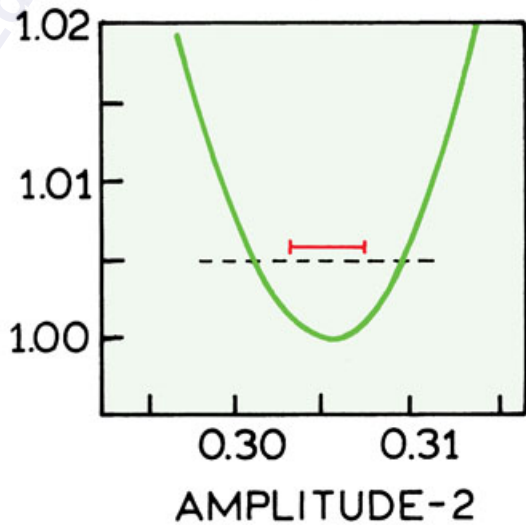
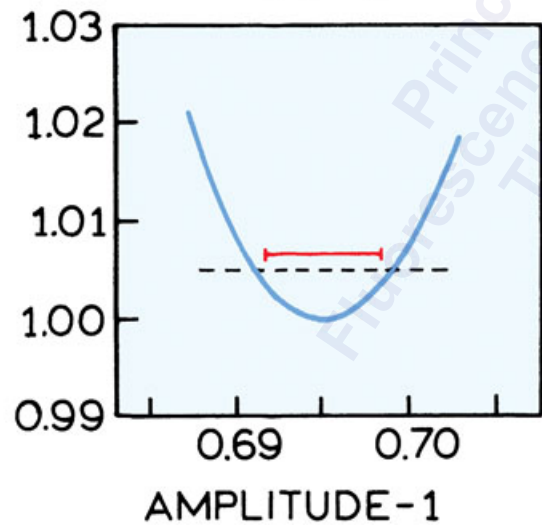
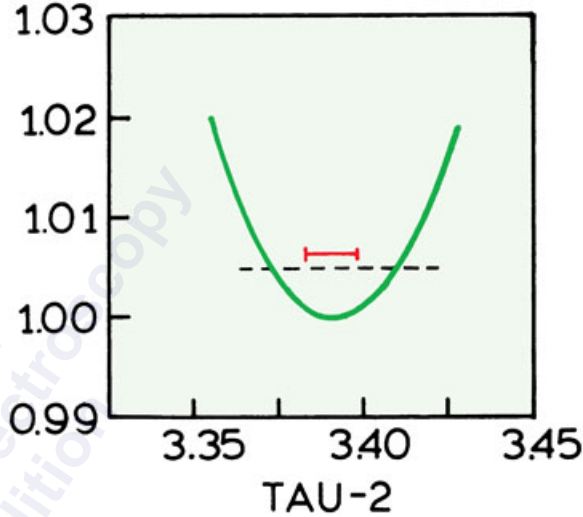
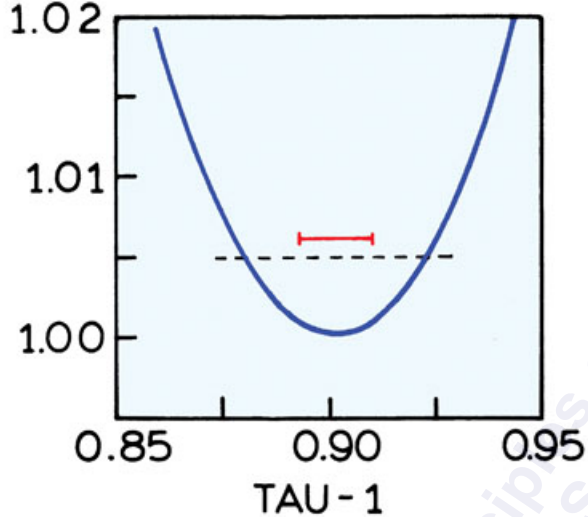


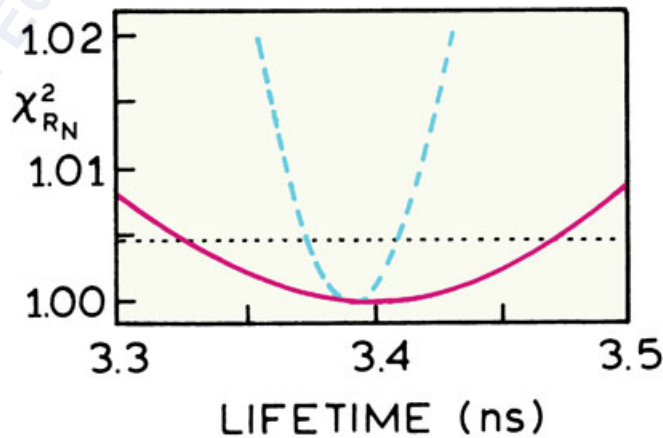
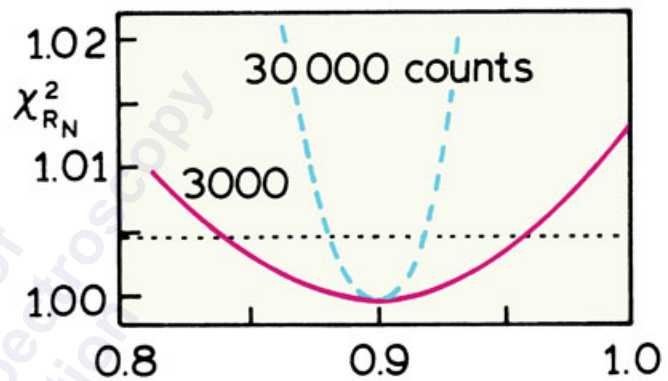
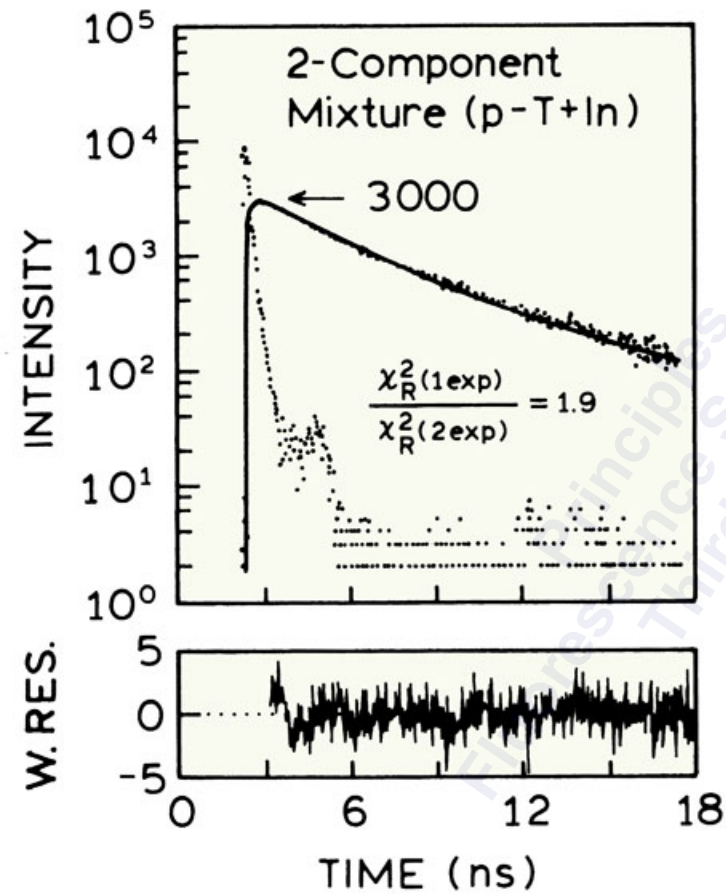
FLUORESCENCE INTENSITY



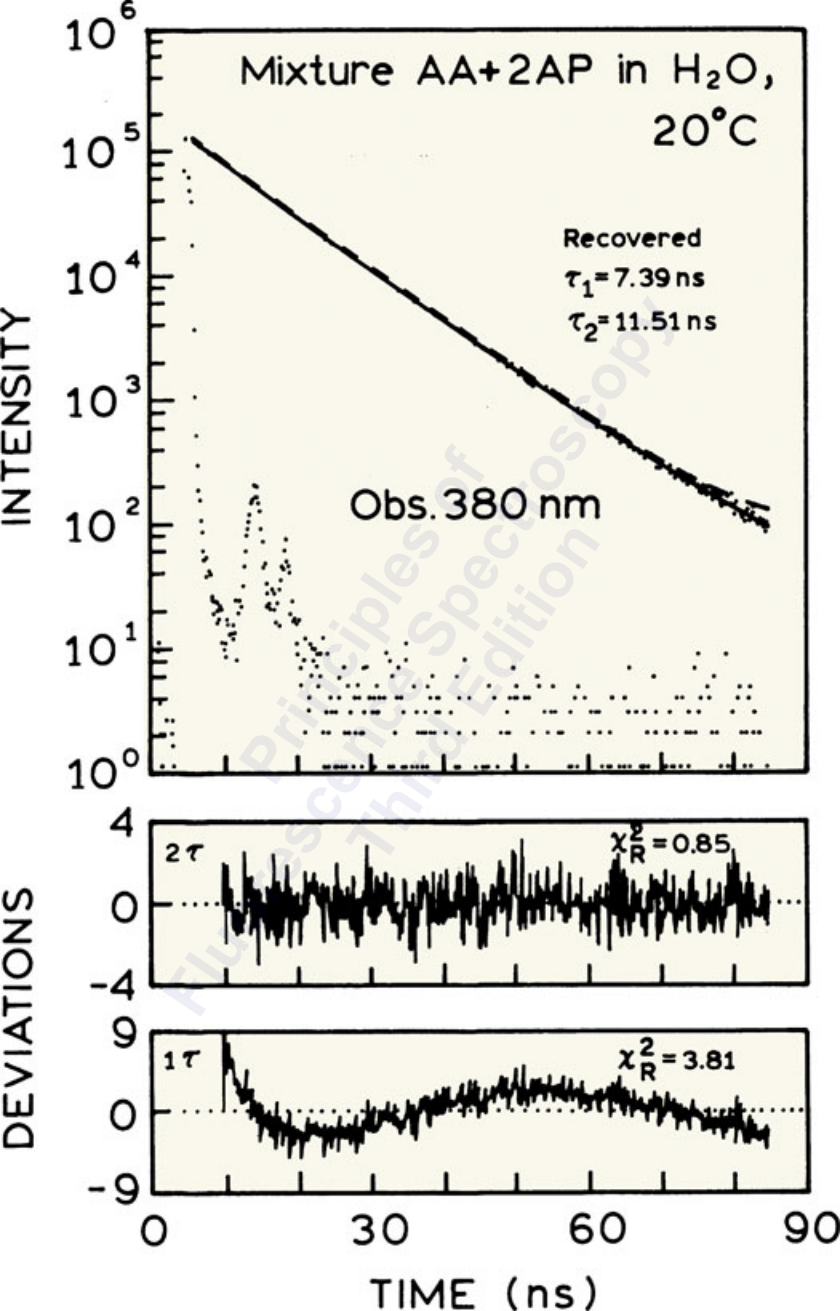


$\chi_R^2 / \chi_R^2 \min$

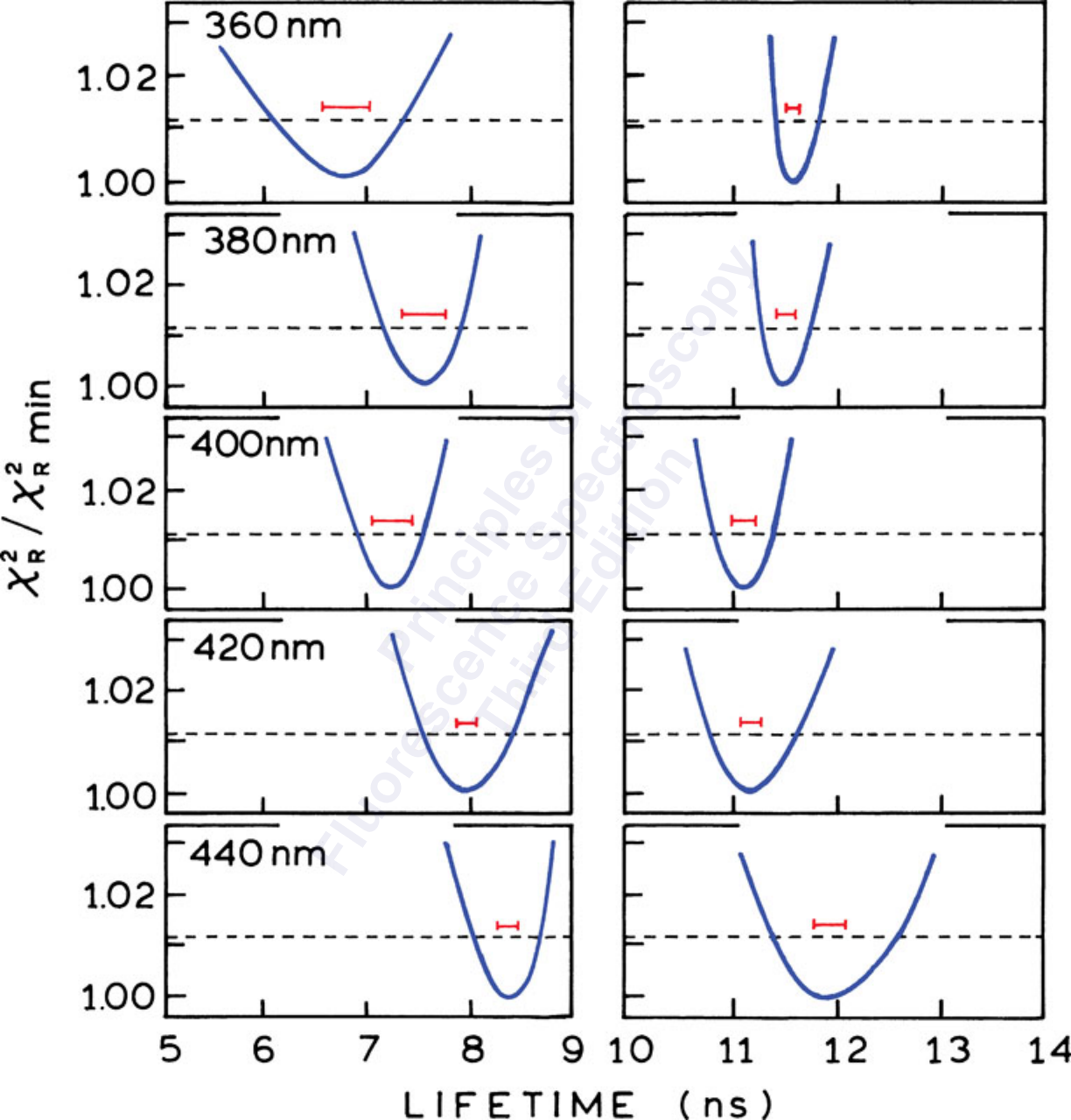


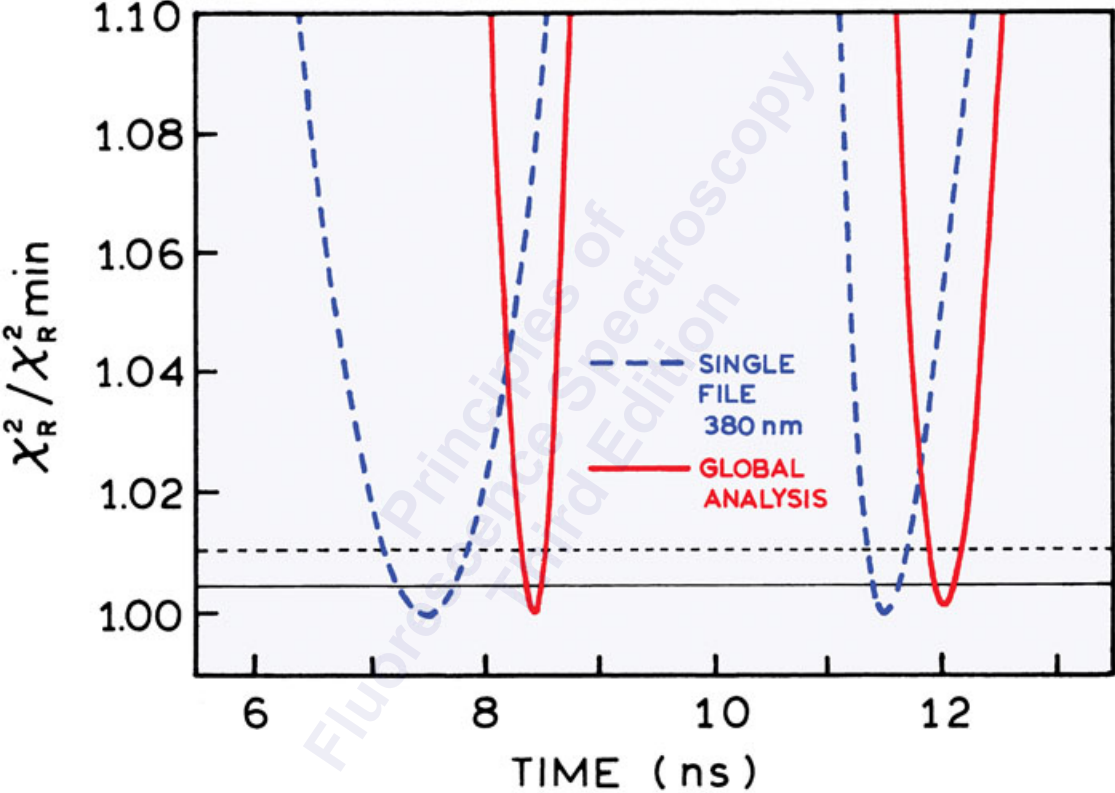


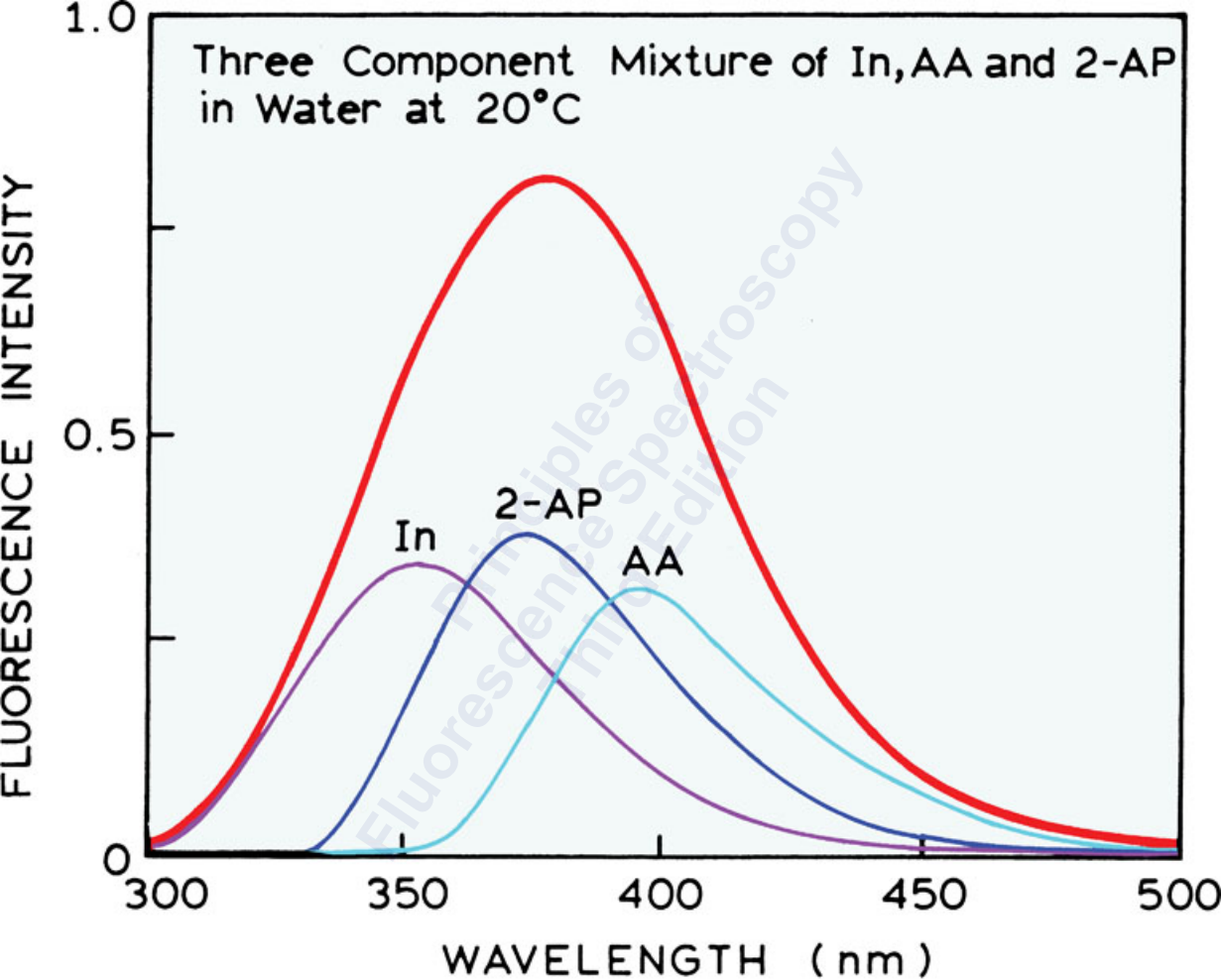


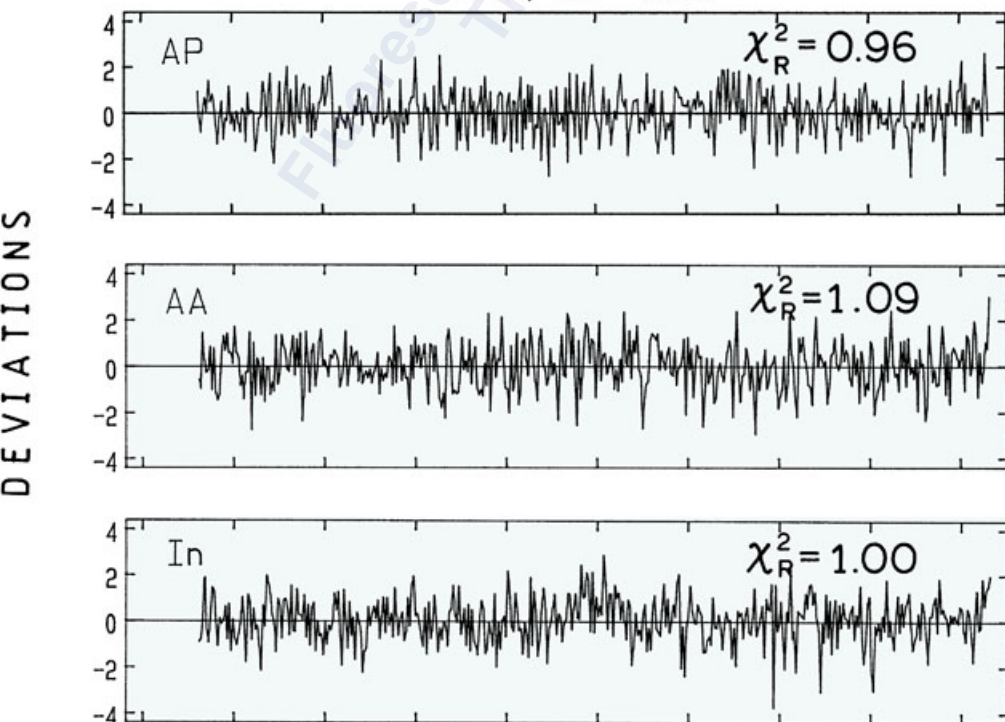
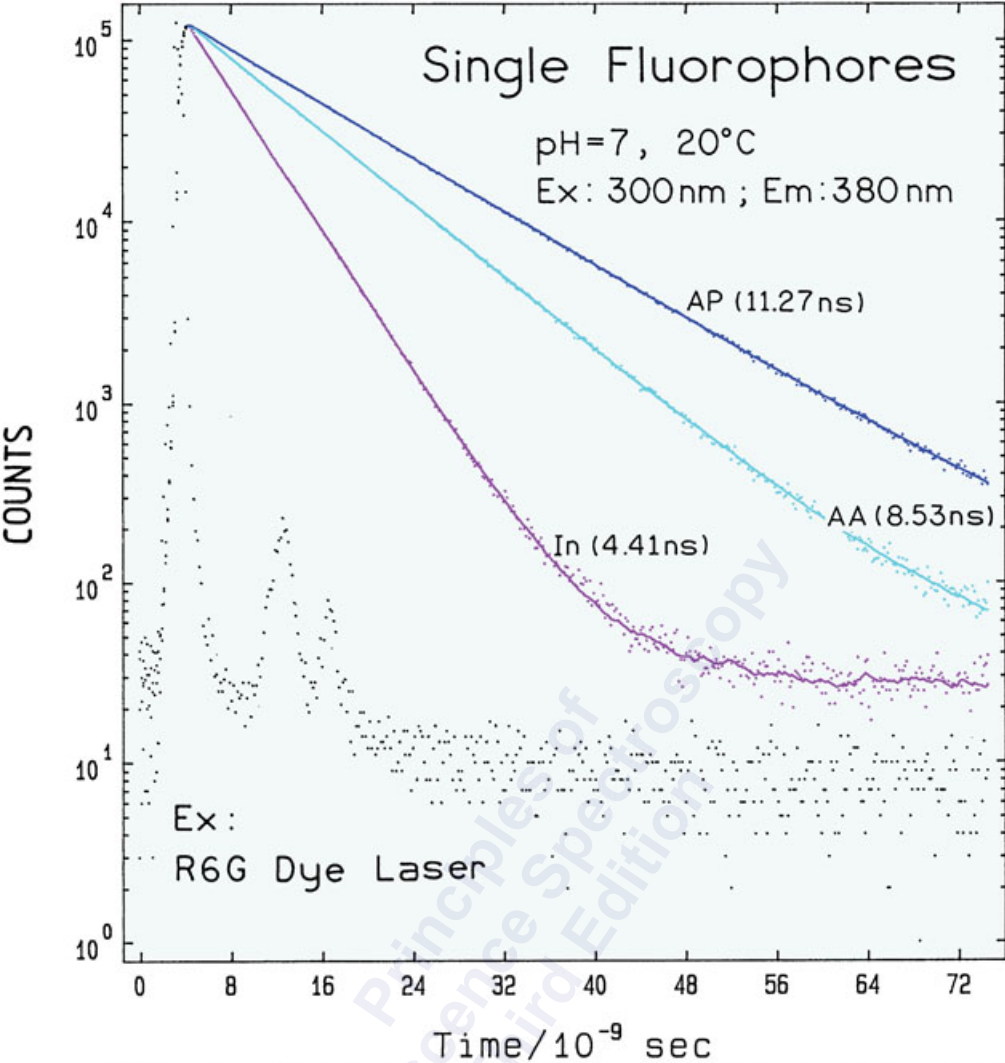


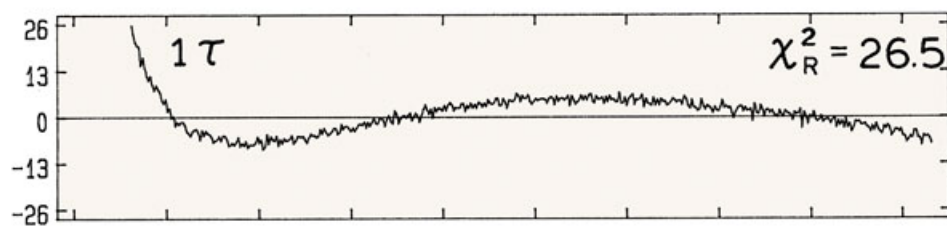
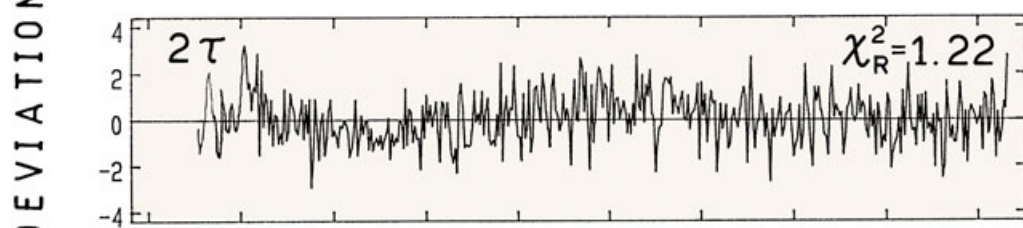
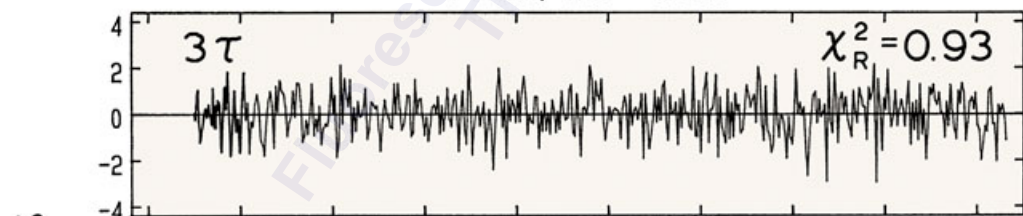
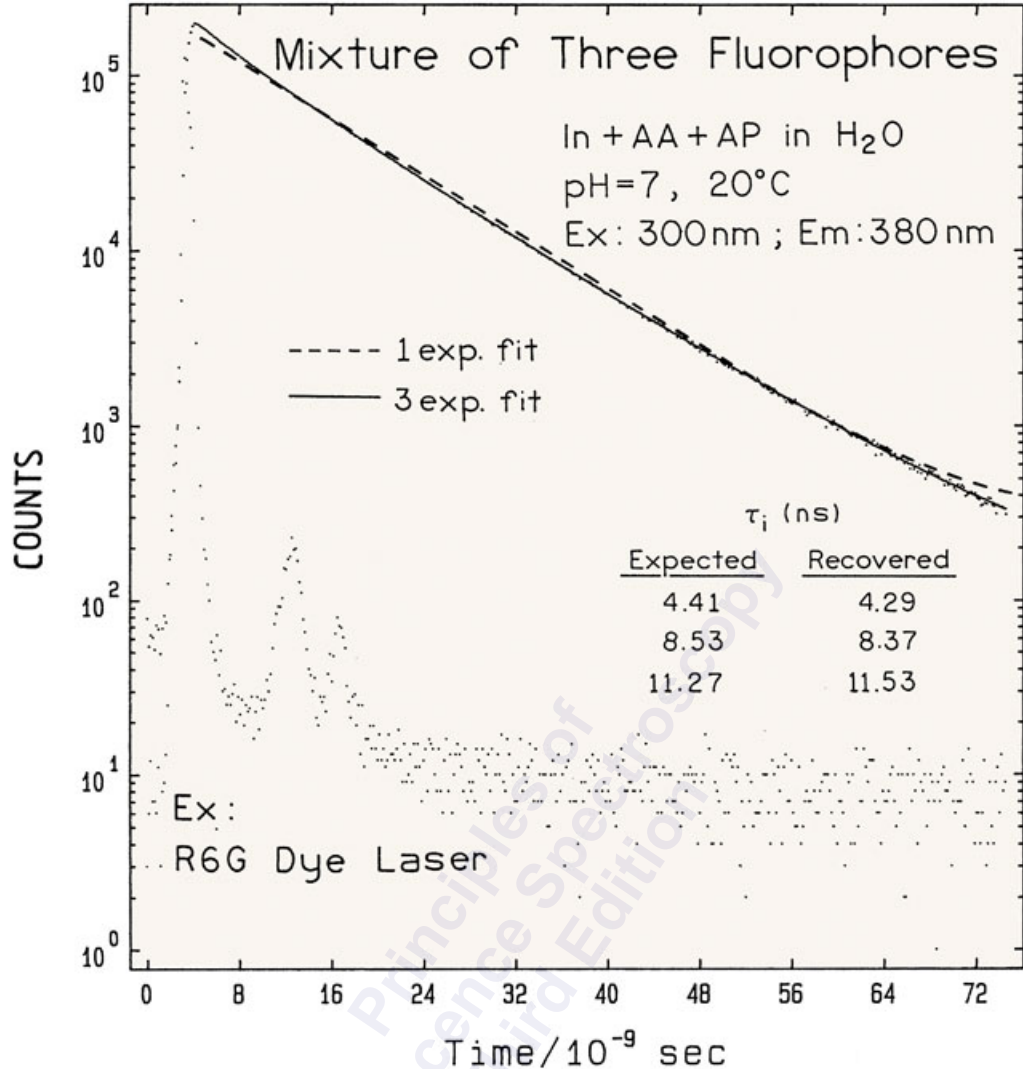


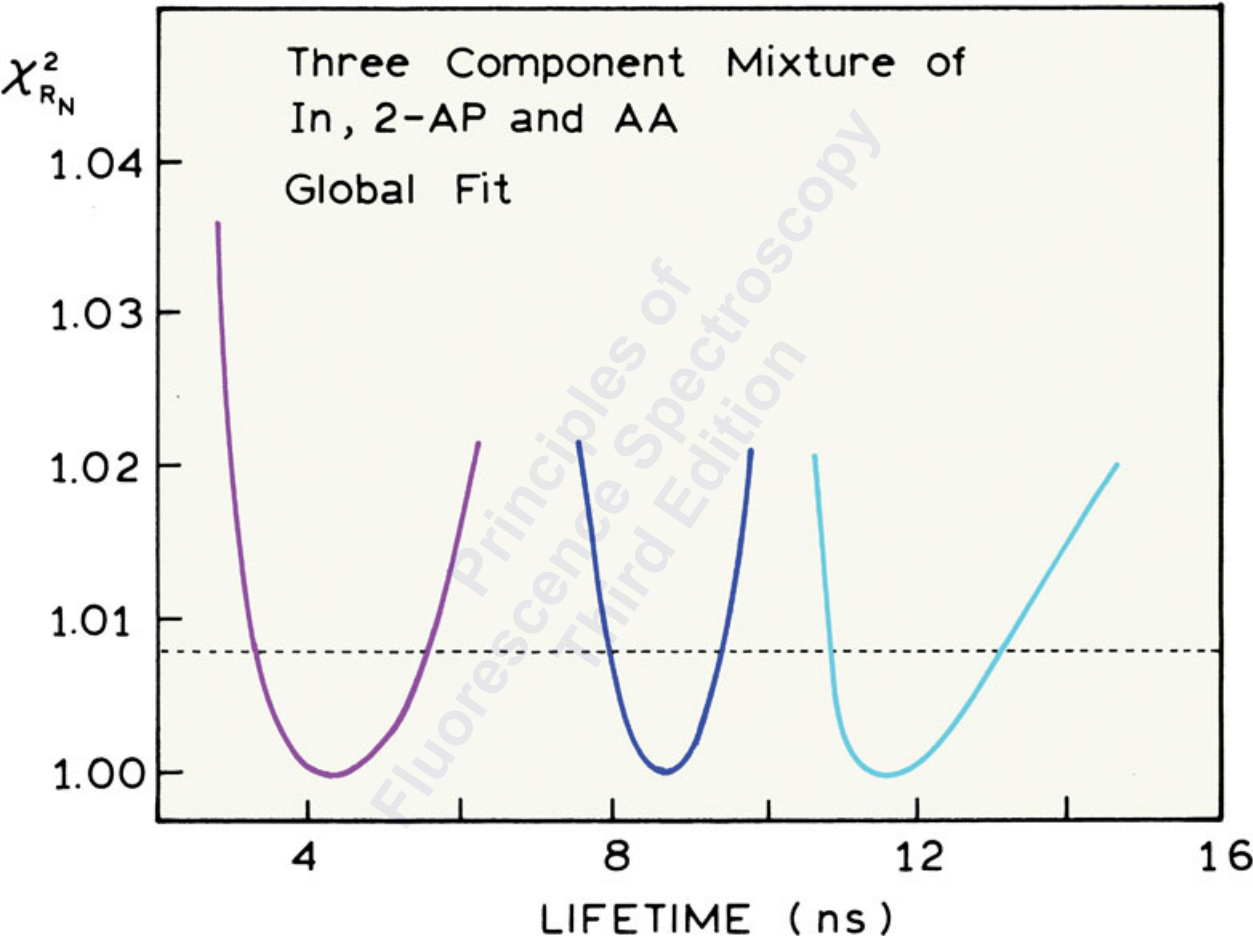






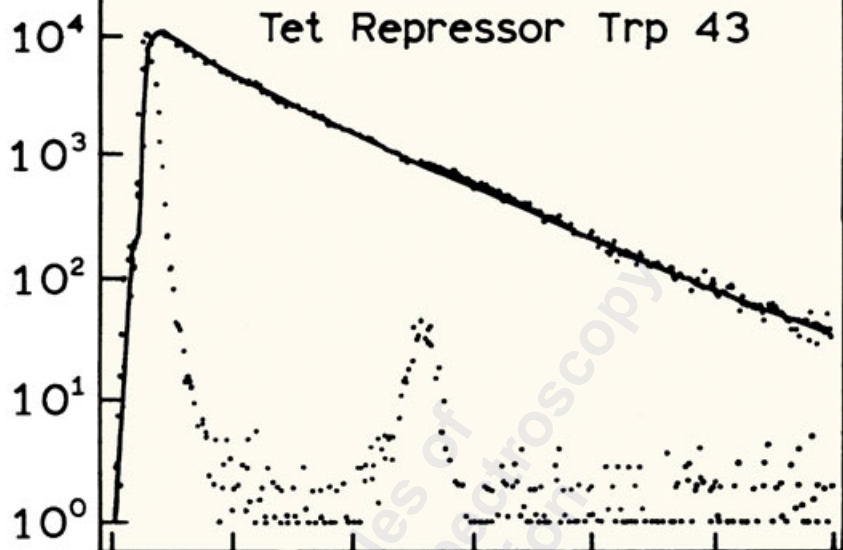




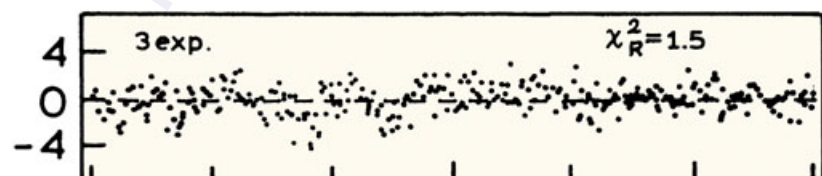
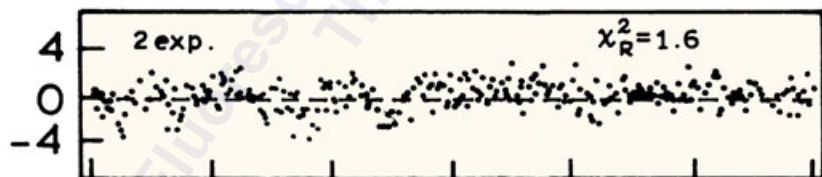
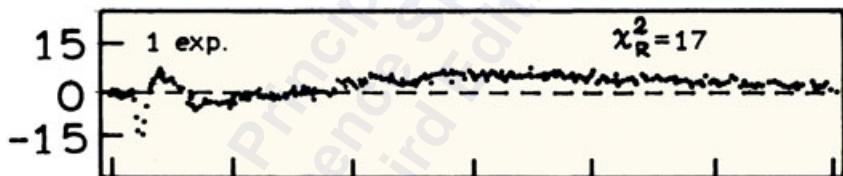




COUNTS



W. RESIDUALS



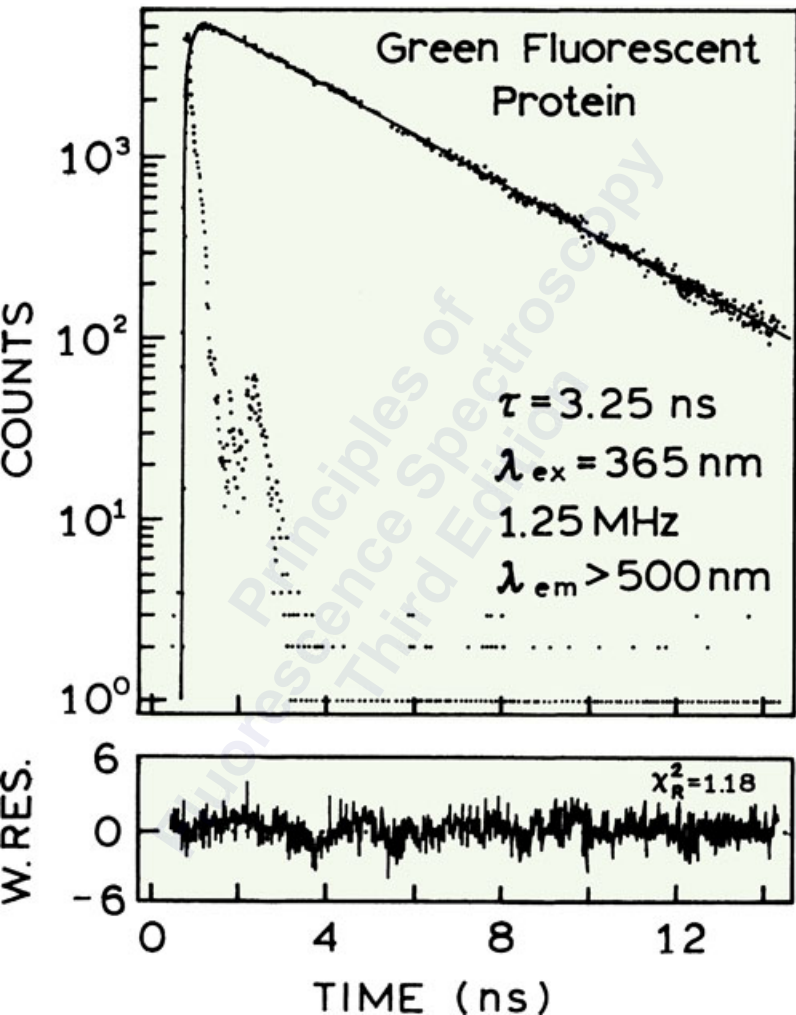
0

100

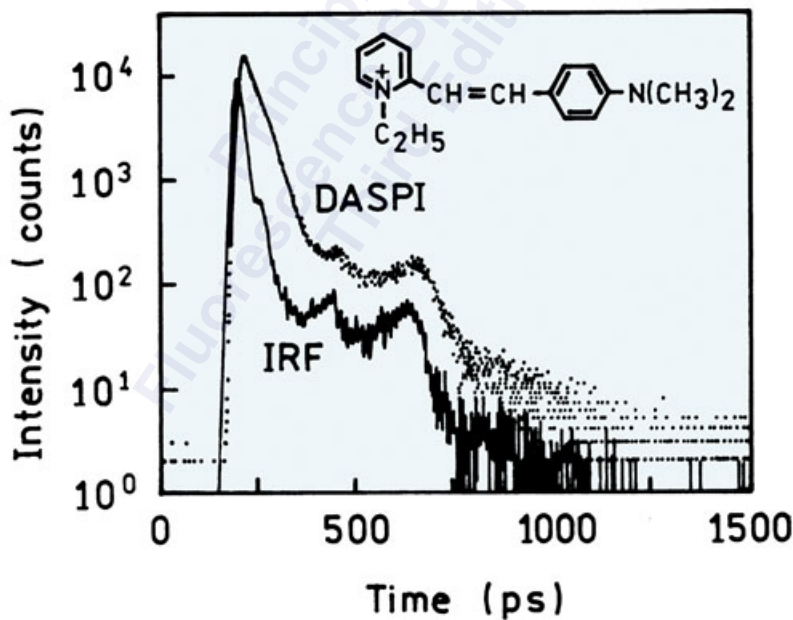
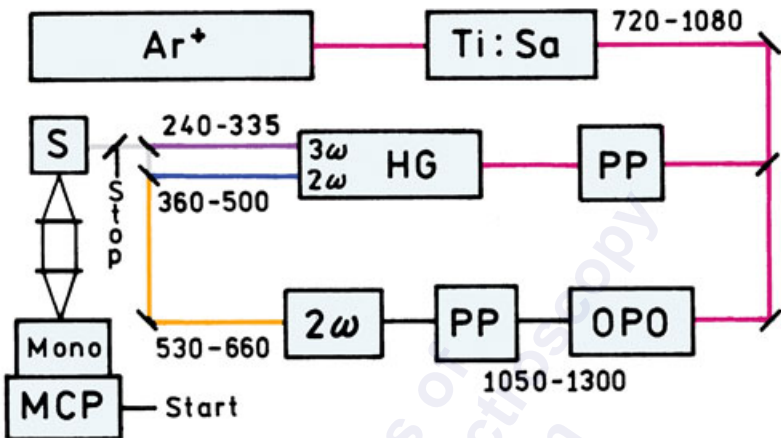
200

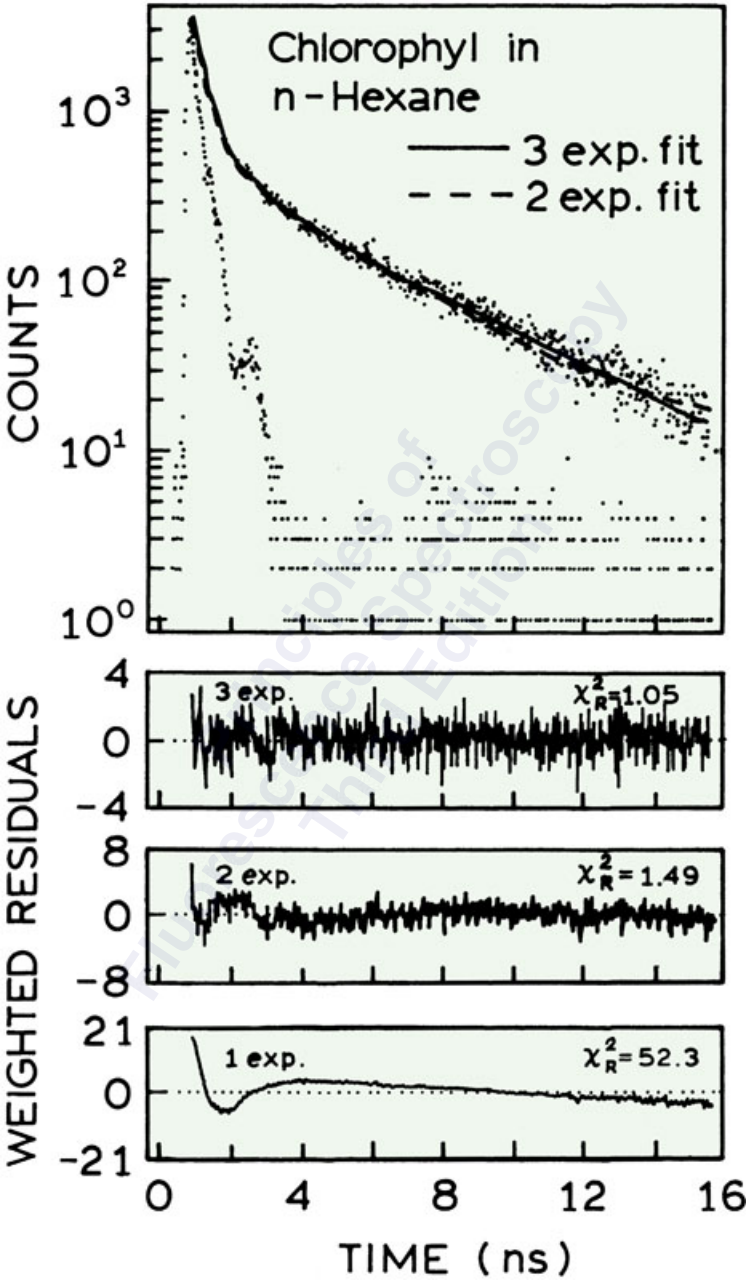
300

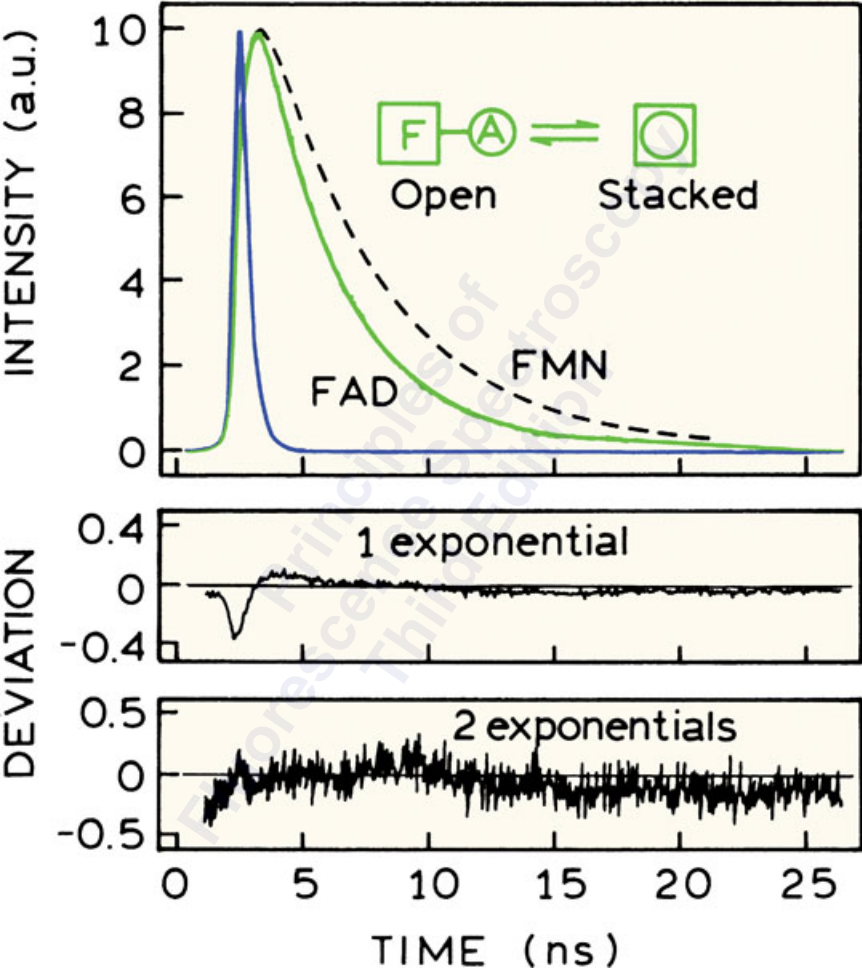
CHANNEL NUMBER

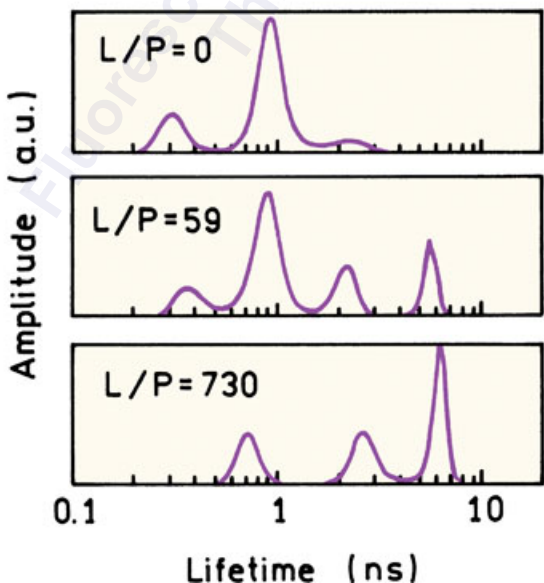
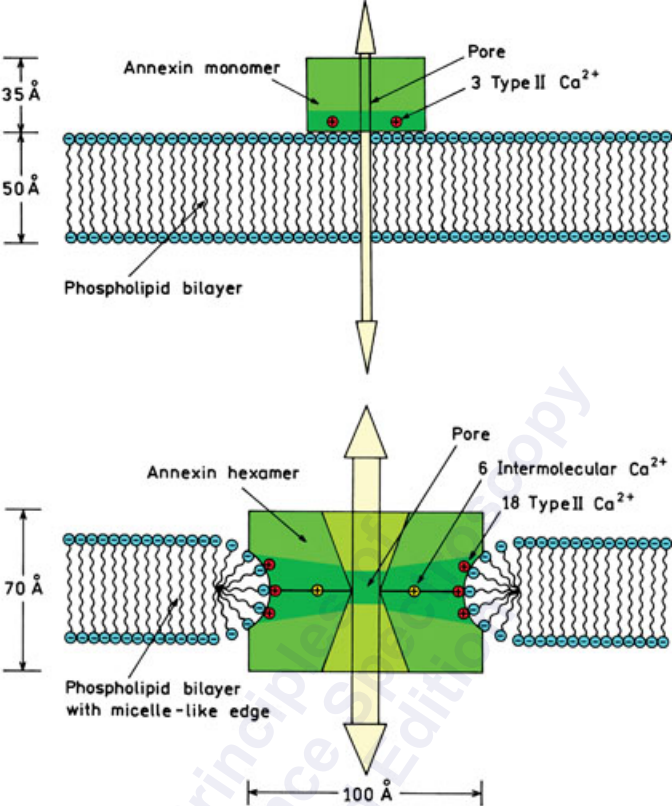


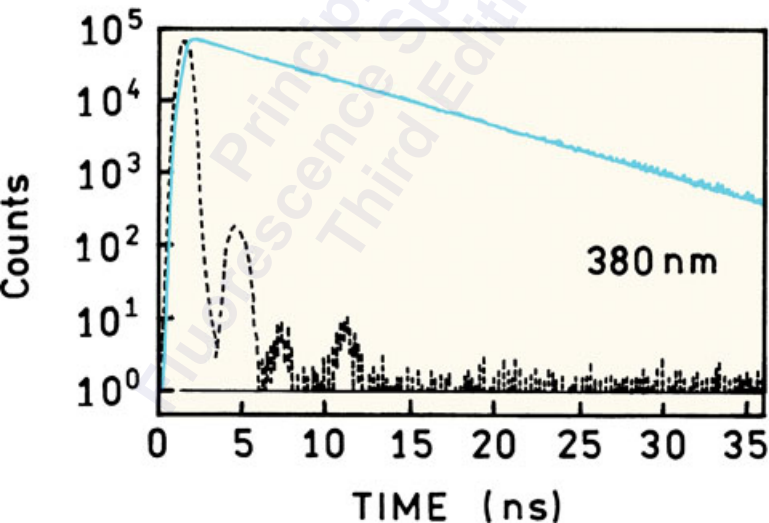
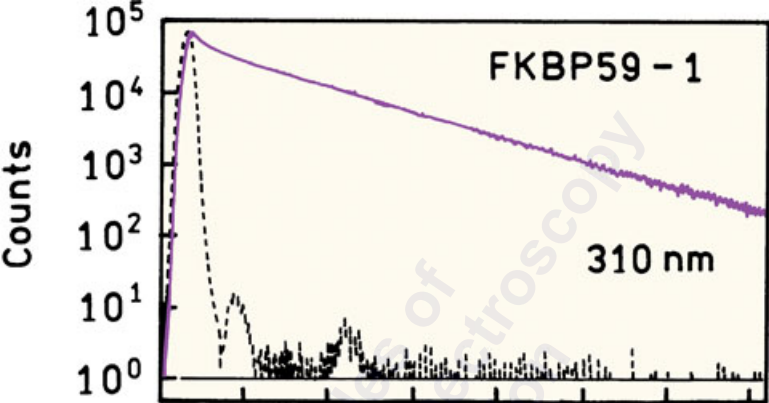




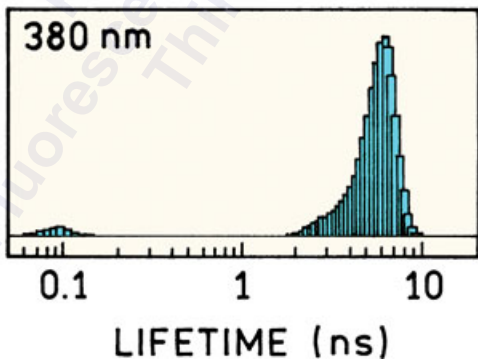
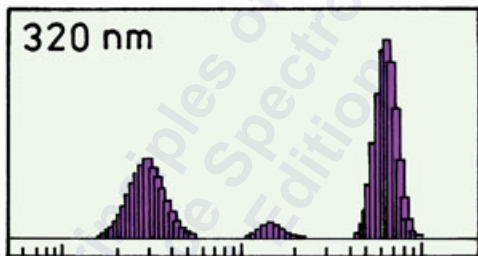
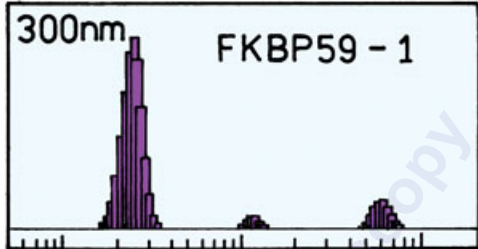


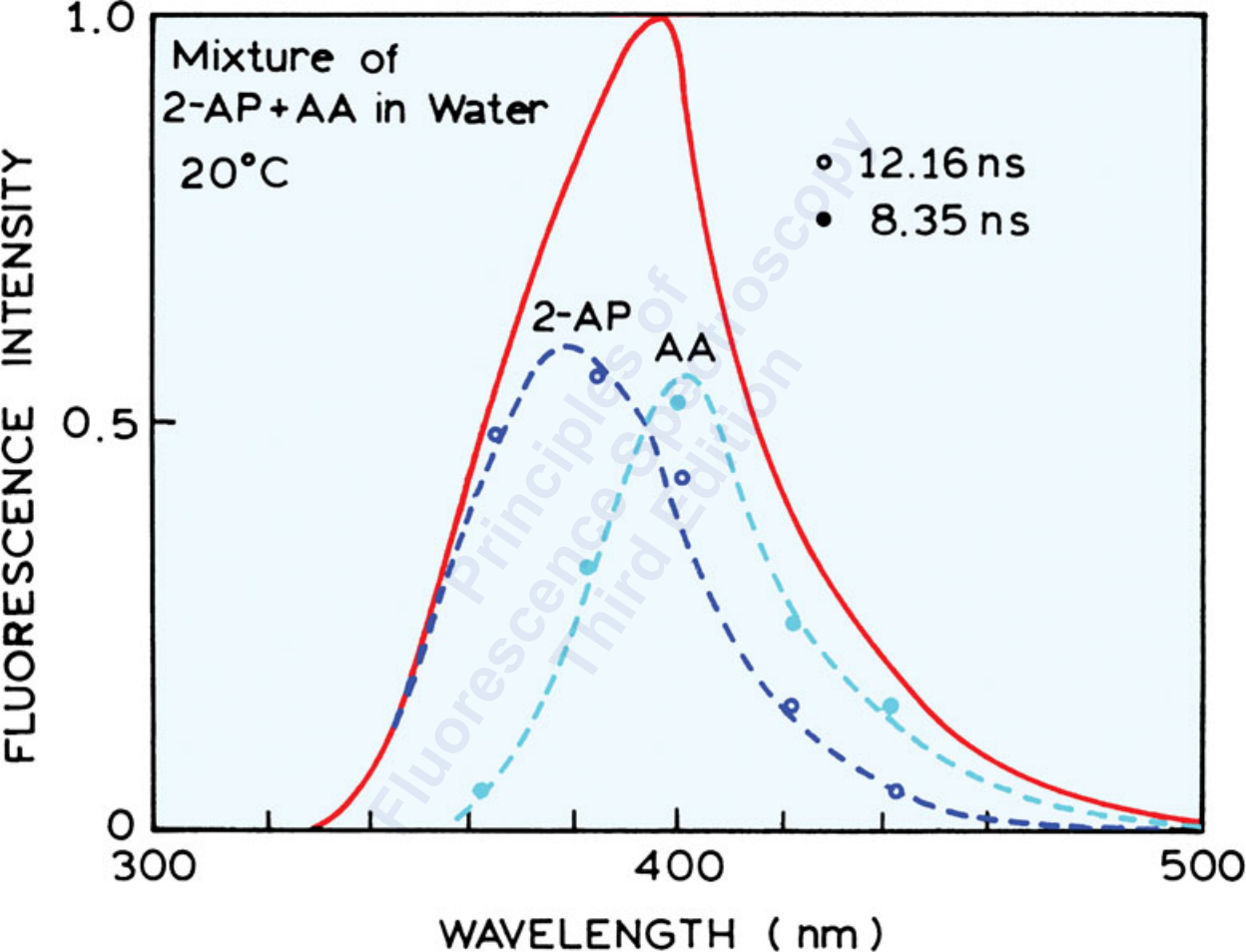


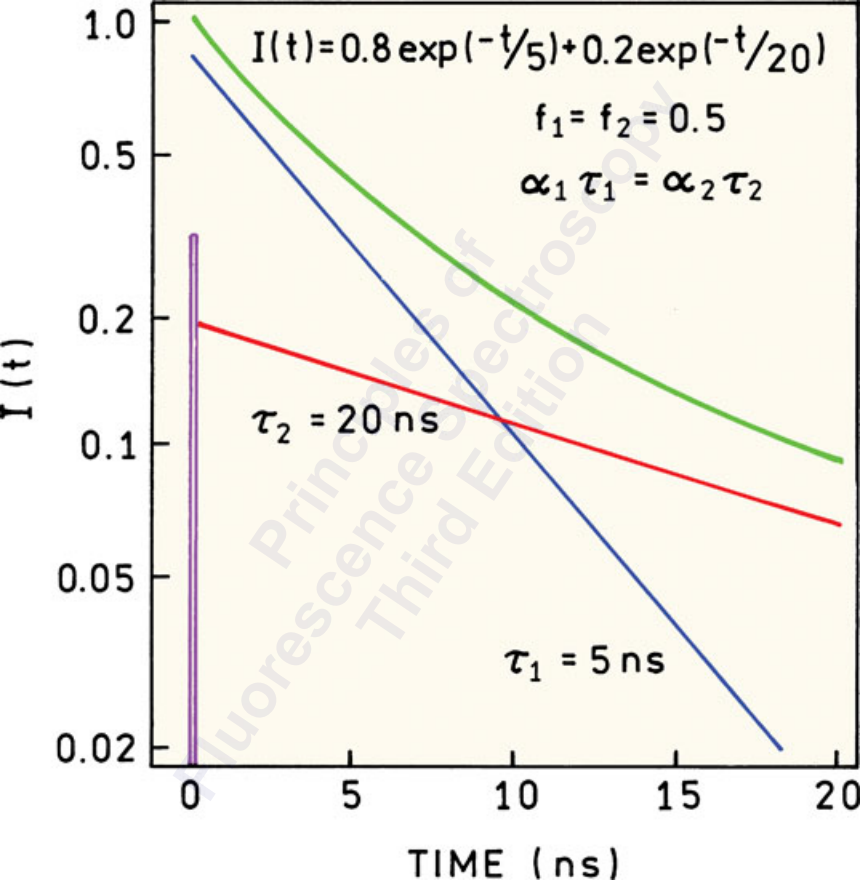




AMPLITUDE (a.u.)

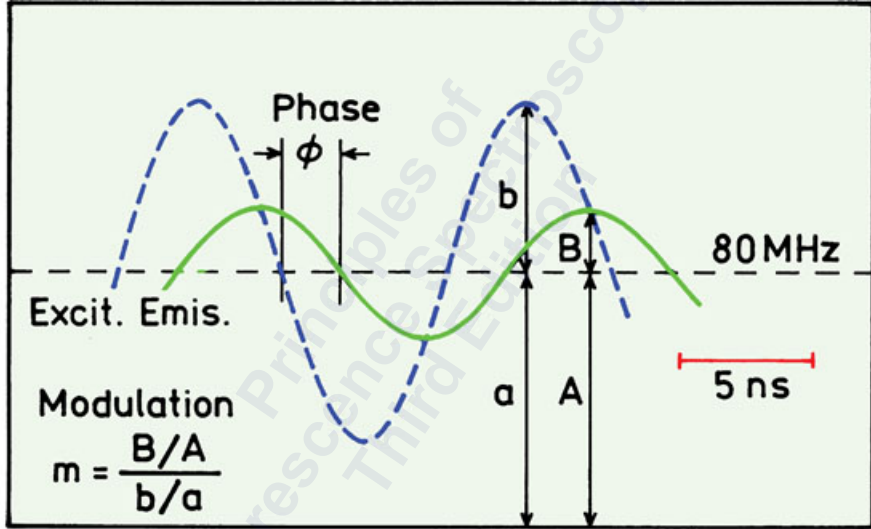




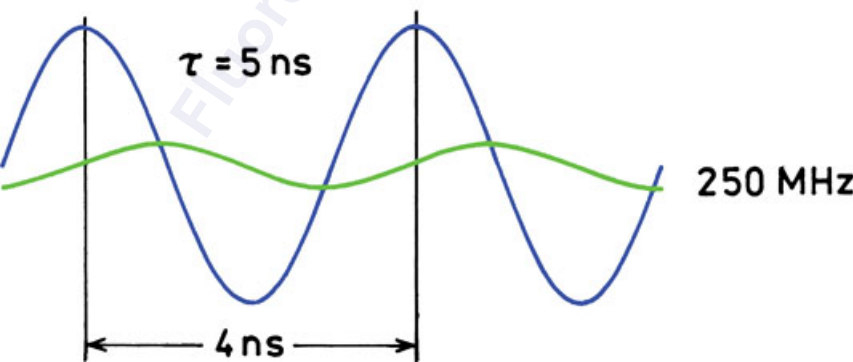
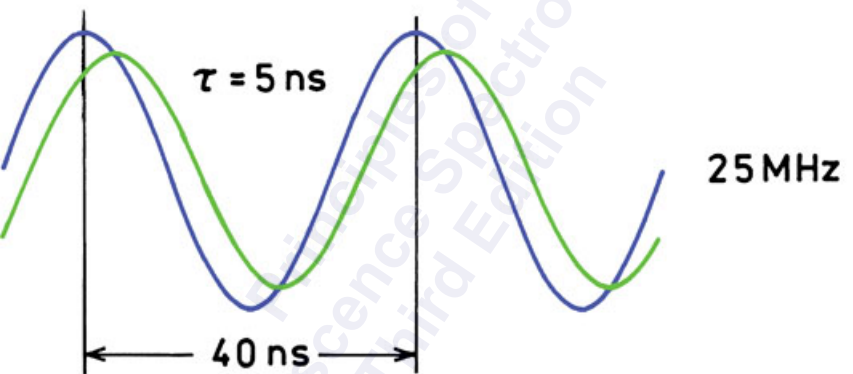
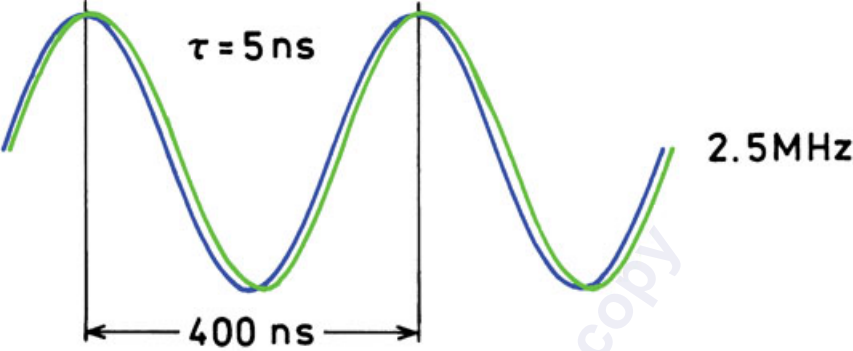


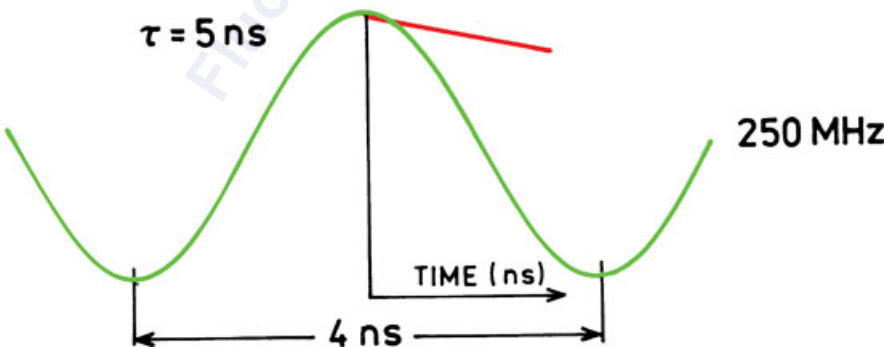
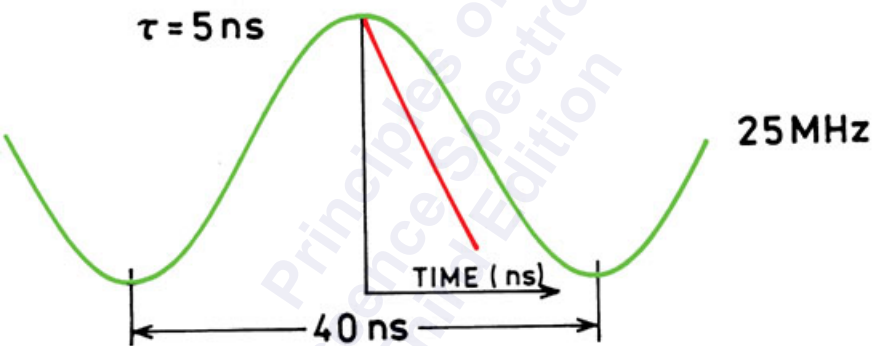
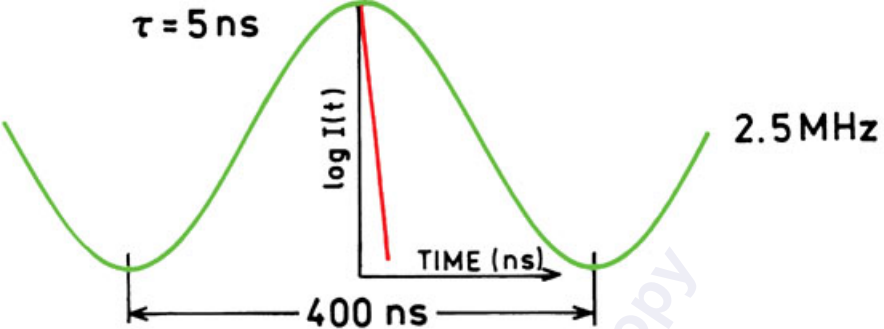


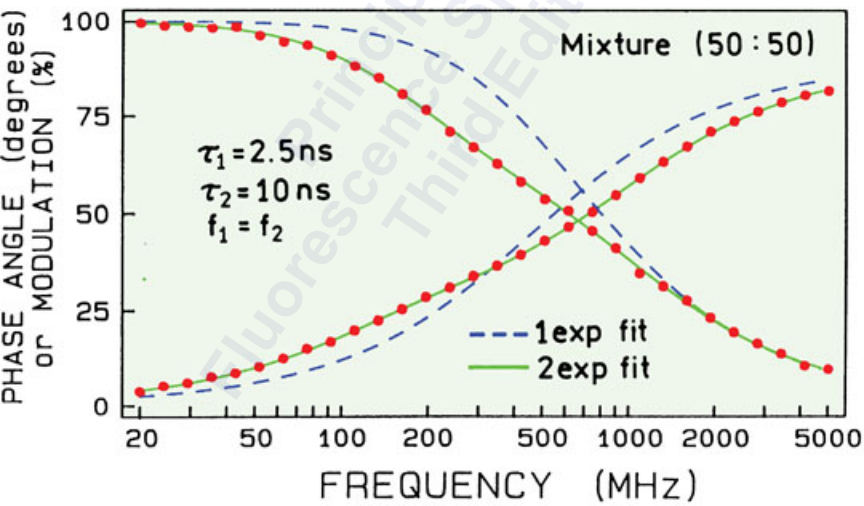
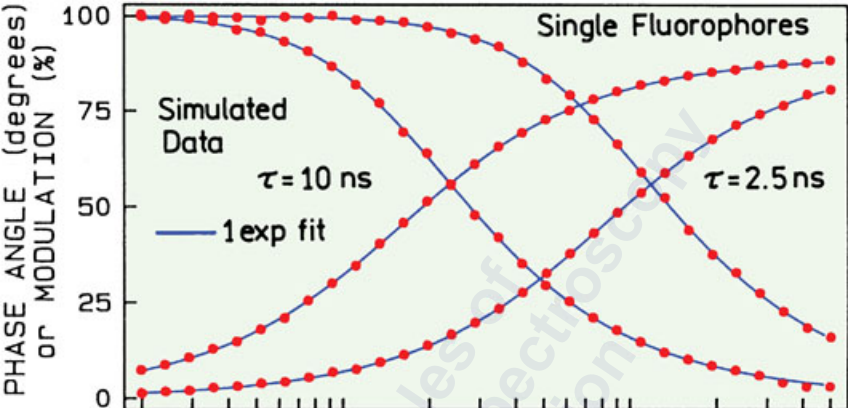
Intensity

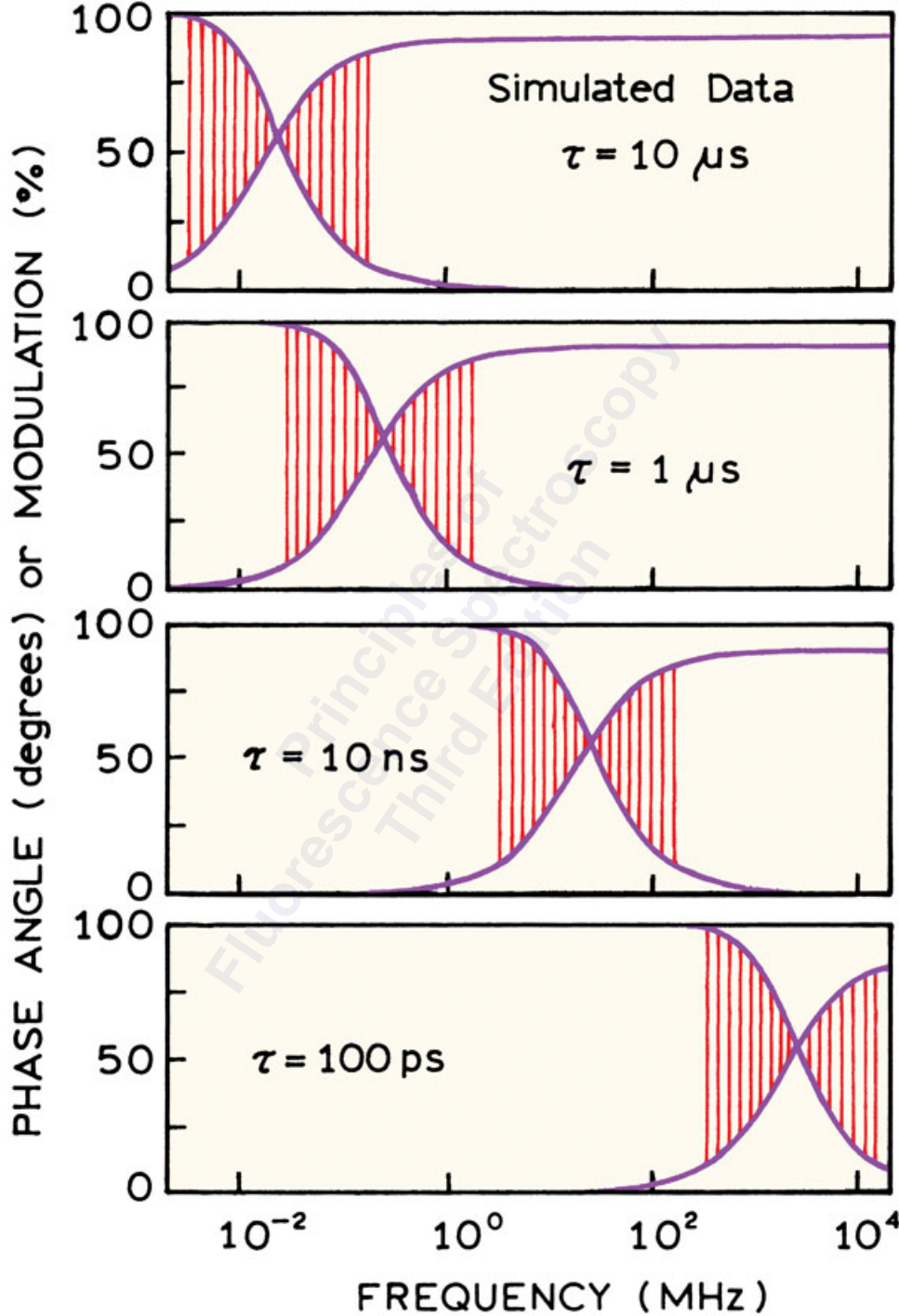


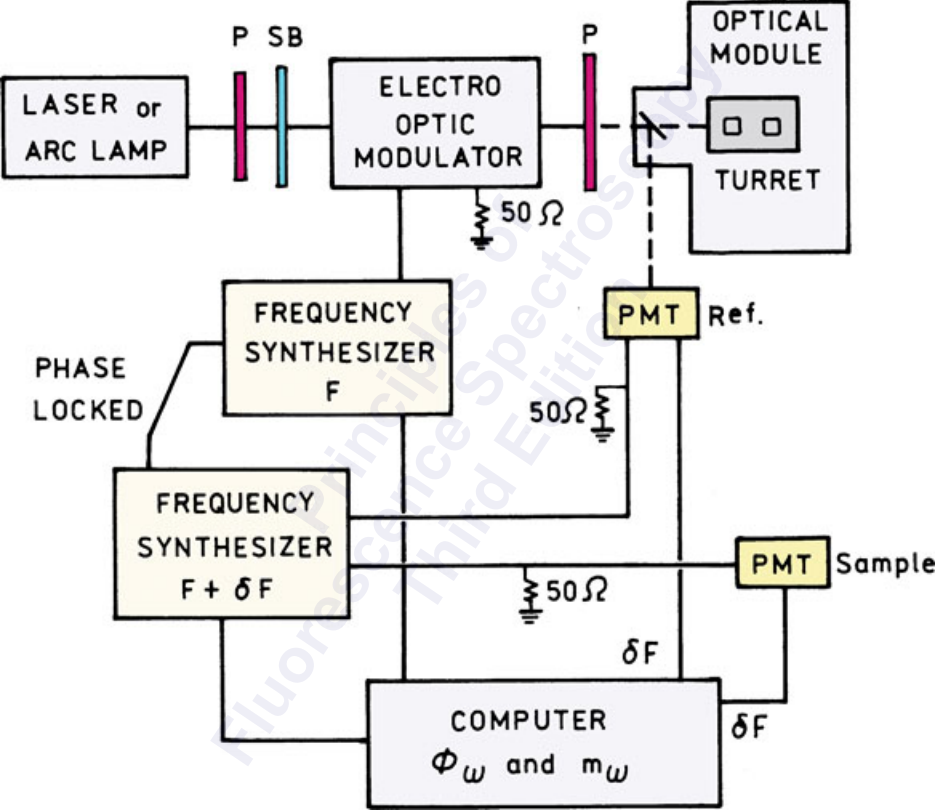
Time

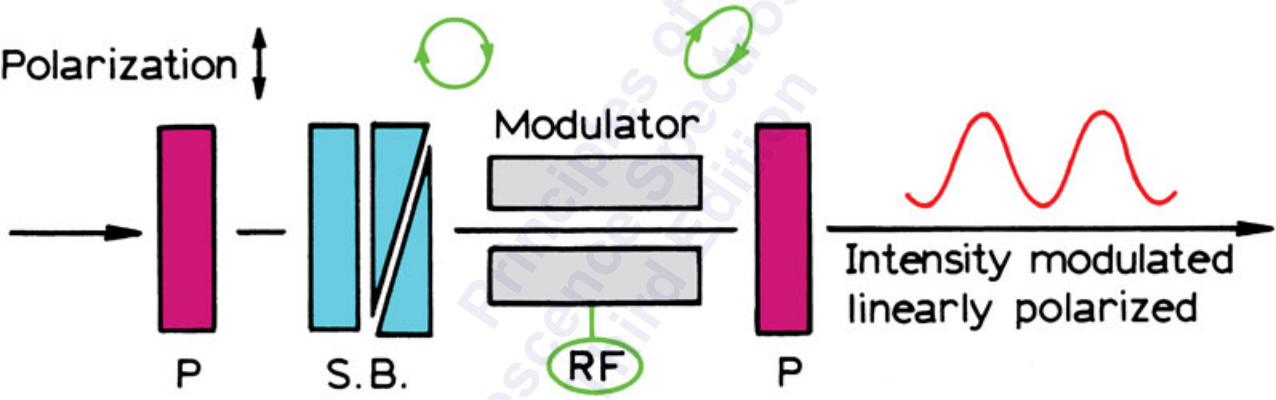


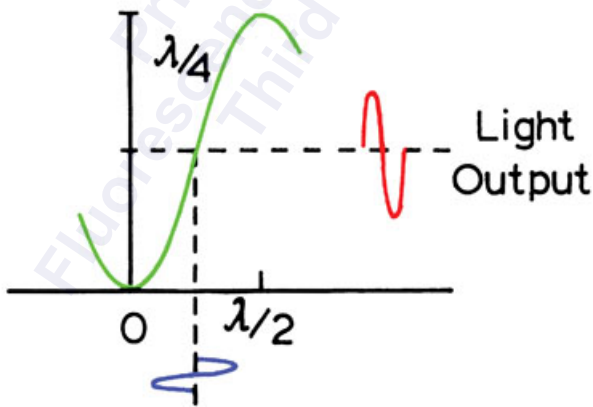
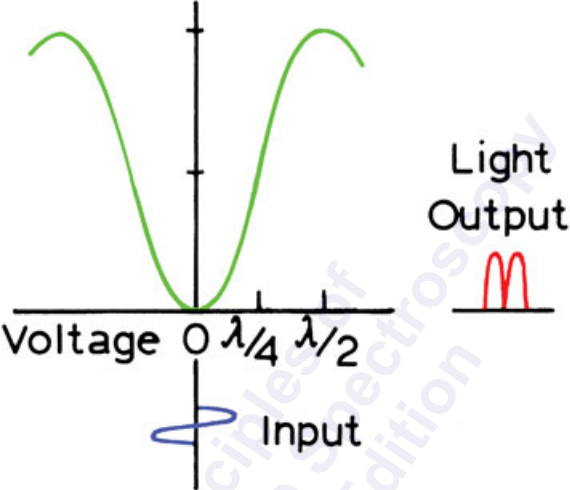






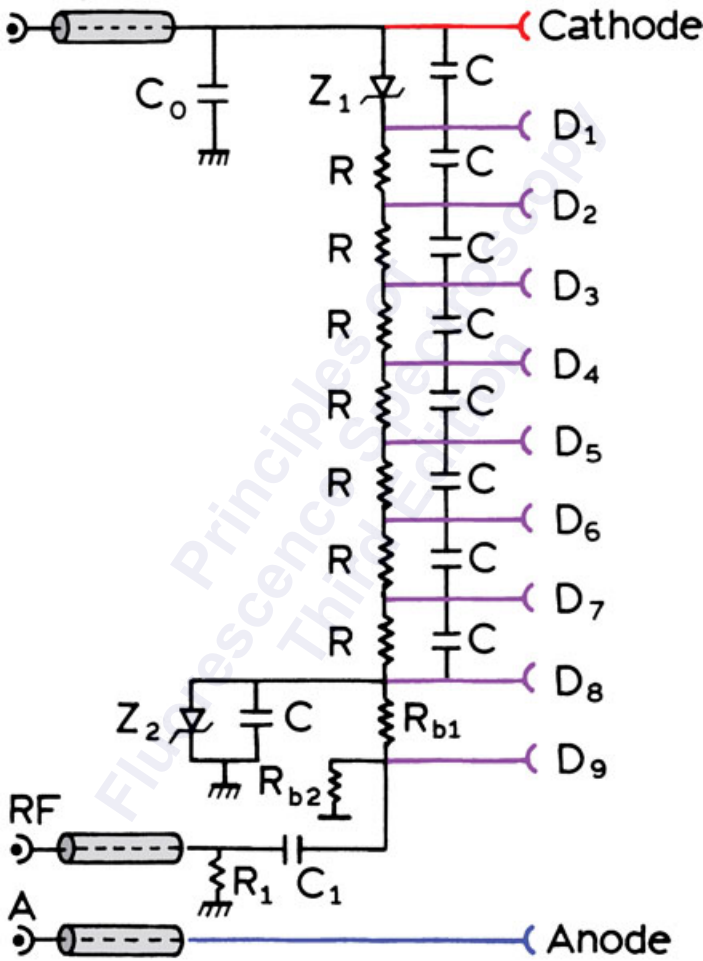








HV (-400-1200V)



Amplitude

1

0

$$\tau = 5 \text{ ns}$$

50 MHz

$$\Phi_{\omega} = \Phi_2 - \Phi_1$$

$\Phi_2$

$\Phi_1$

$\Phi_{\omega}$

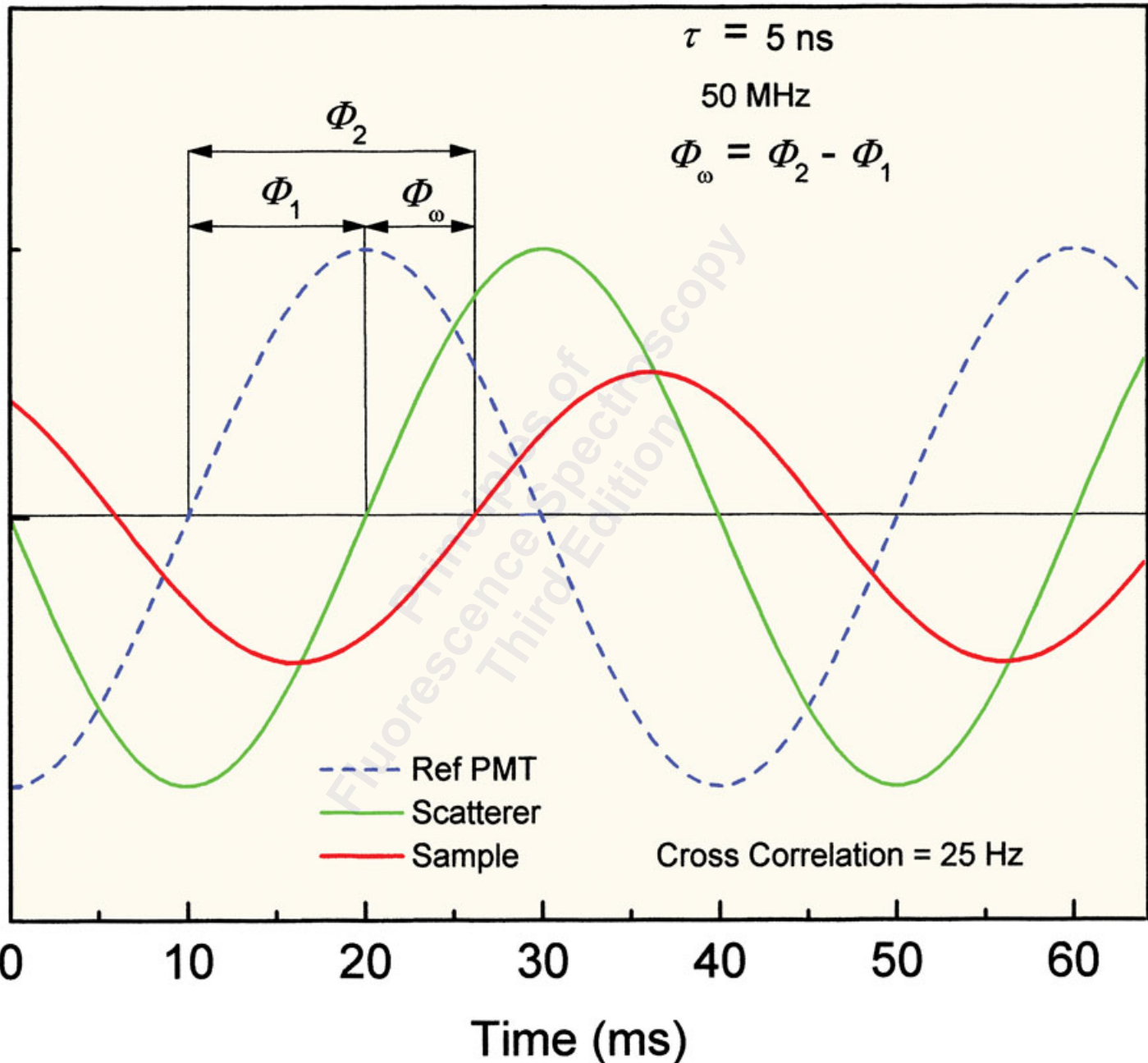
- Ref PMT
- Scatterer
- Sample

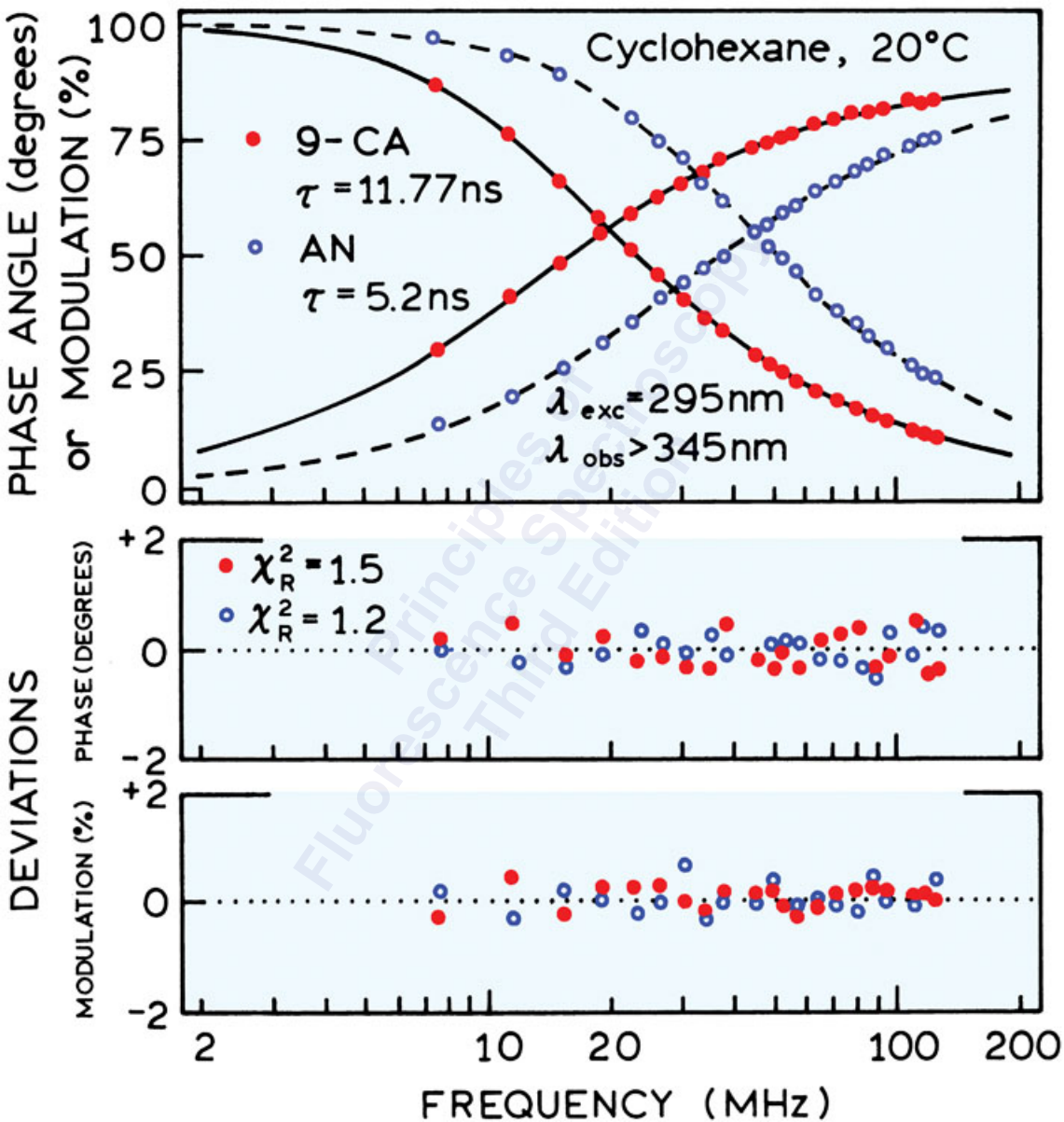
Cross Correlation = 25 Hz

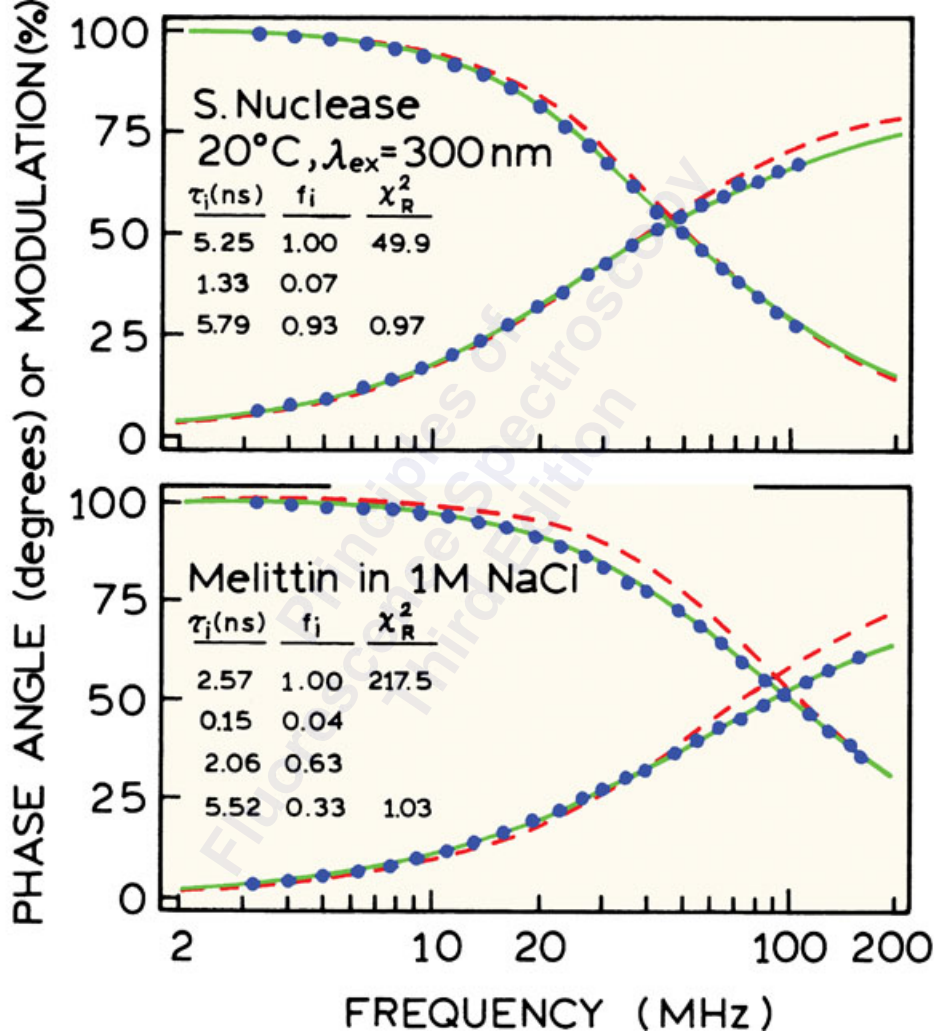
Time (ms)

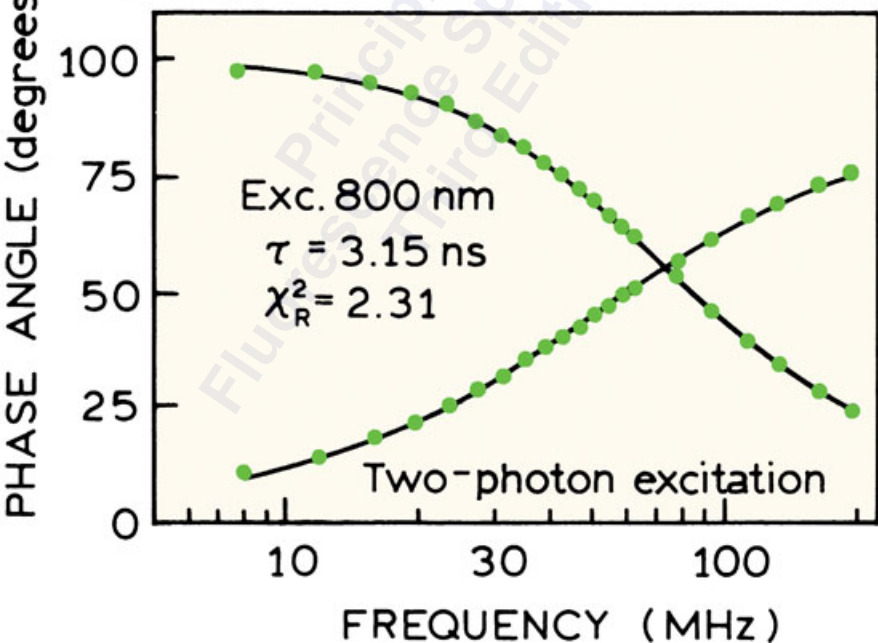
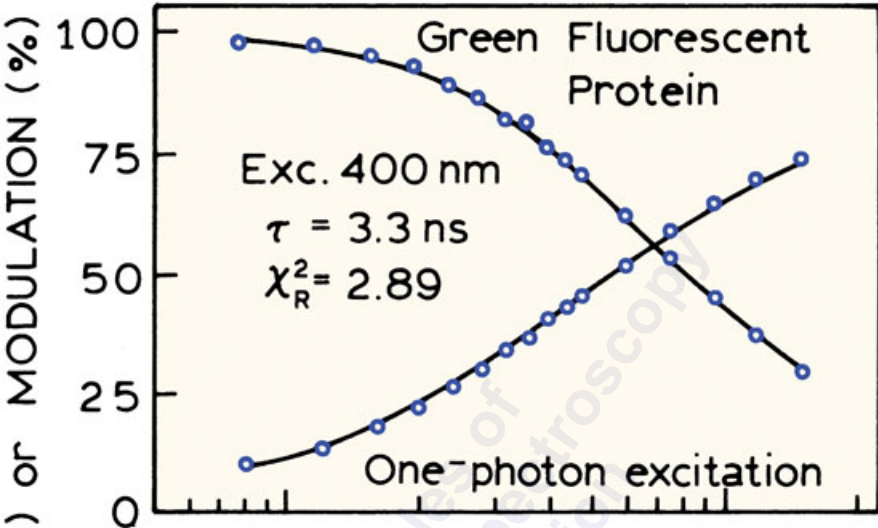
0 10 20 30 40 50 60

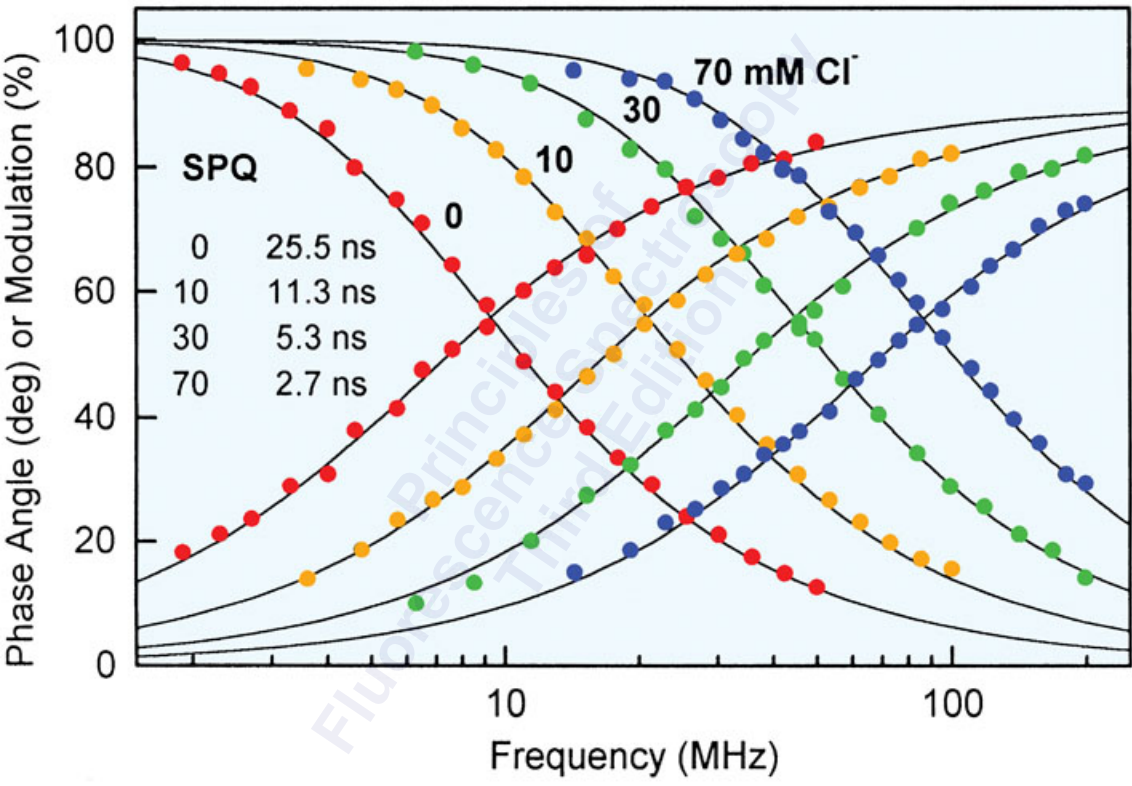
Principles of  
Fluorescence Spectroscopy  
Third Edition



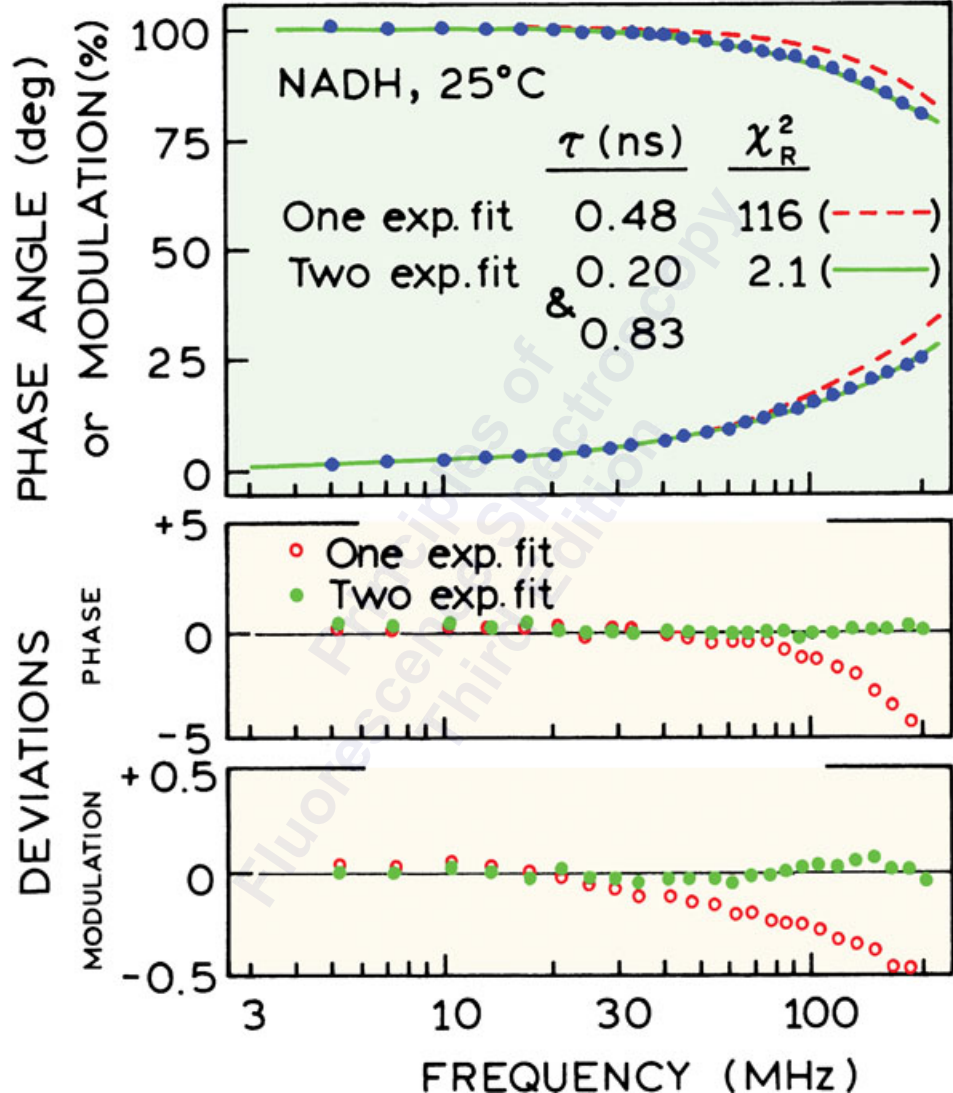


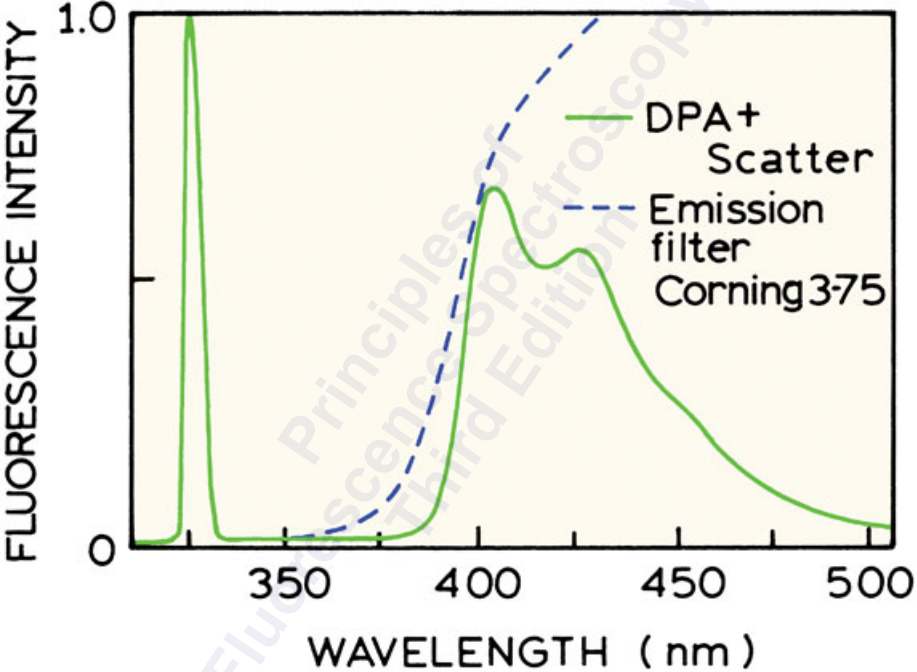




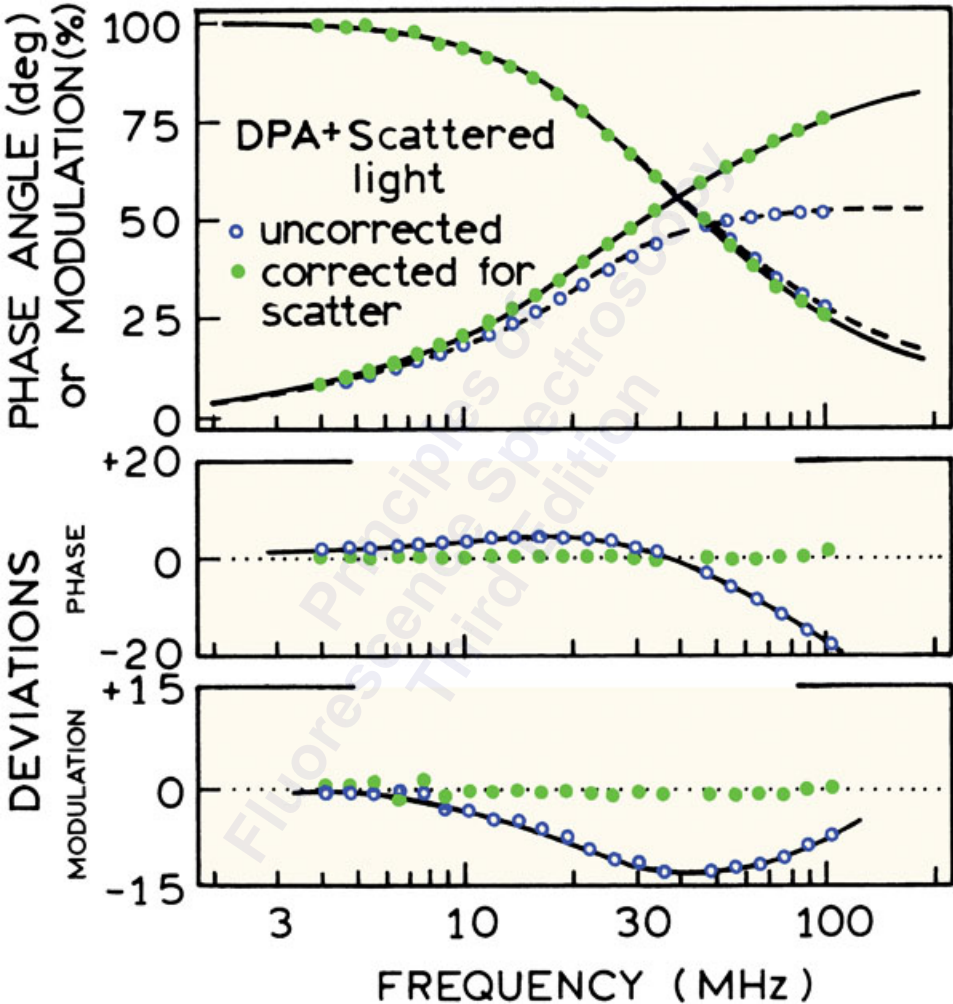


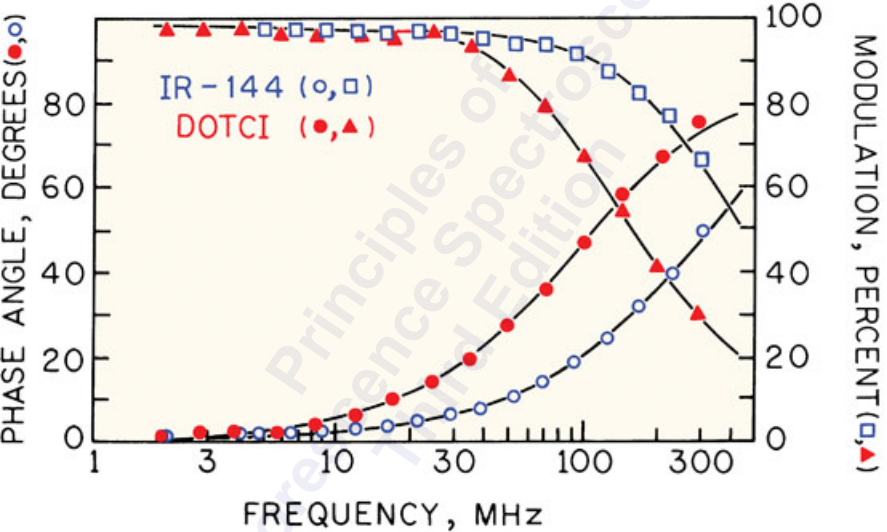


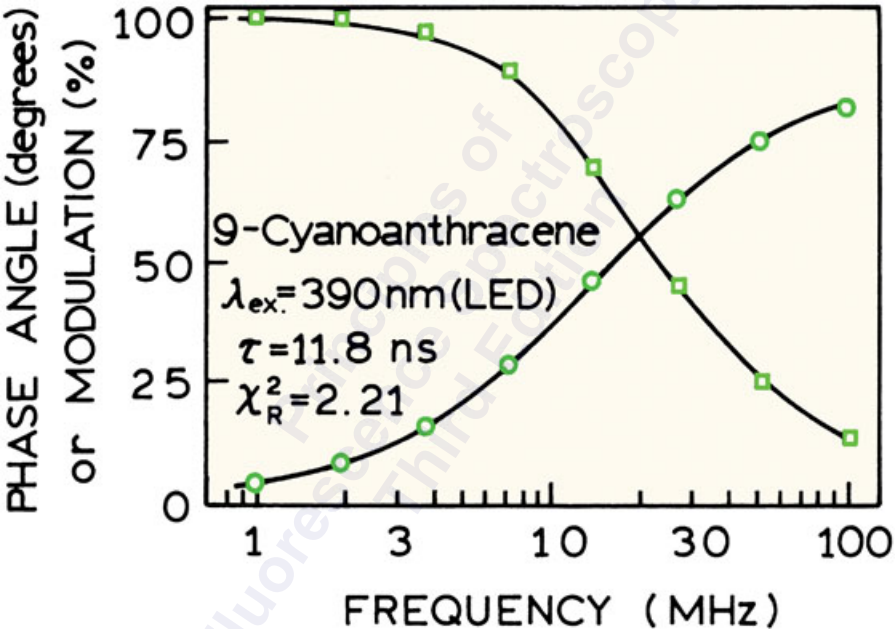


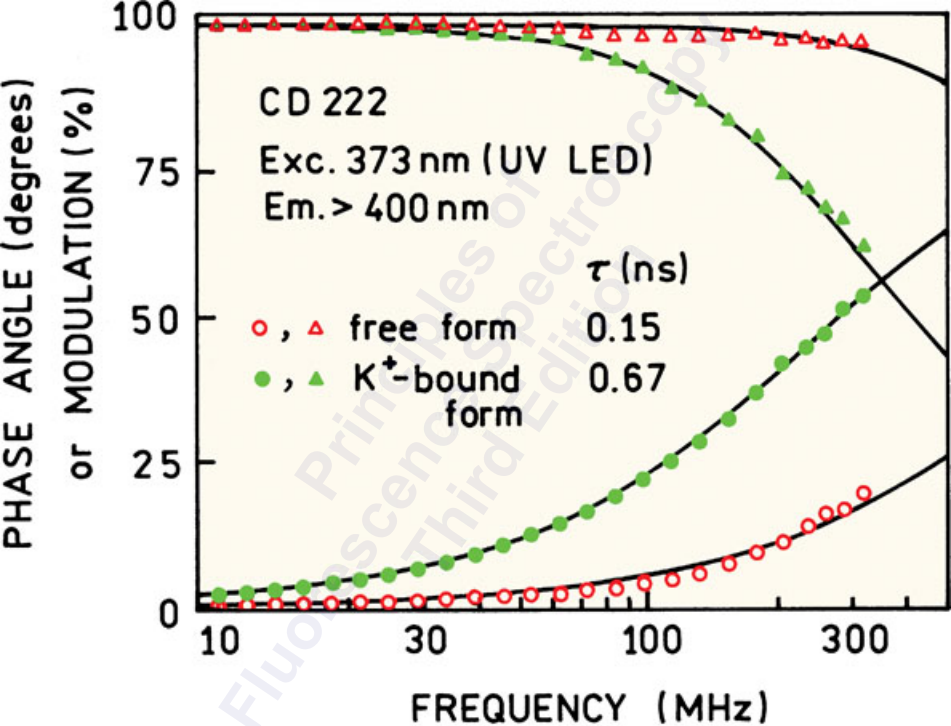




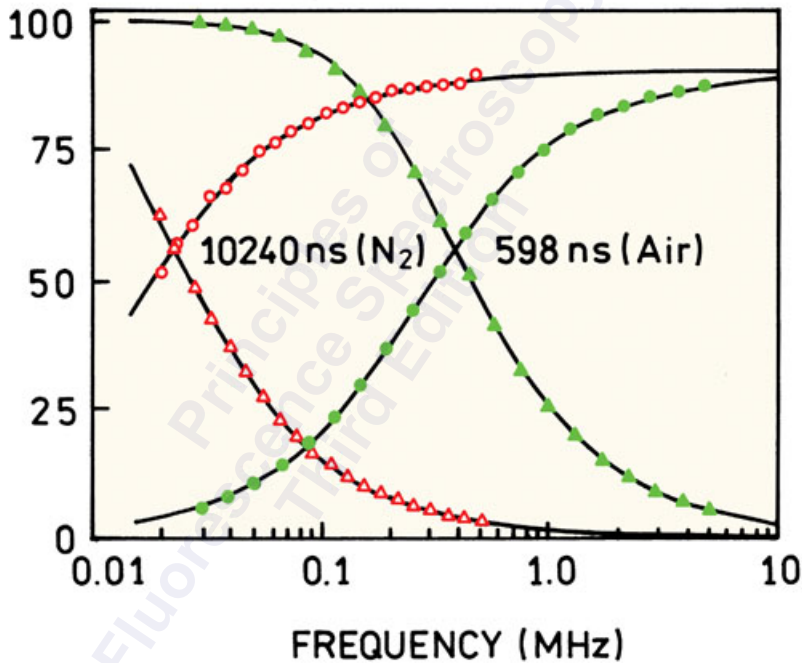


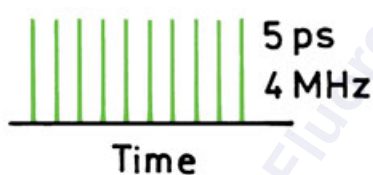
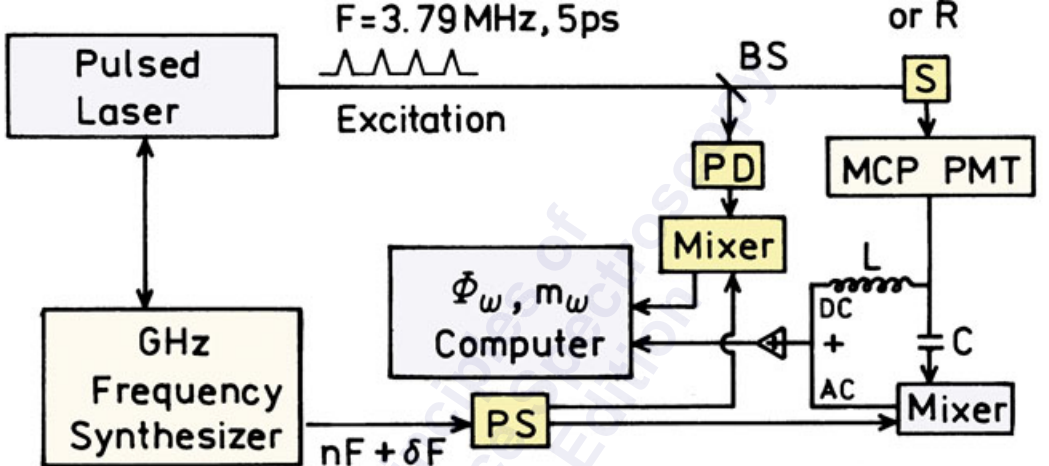




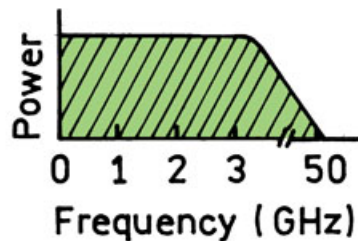


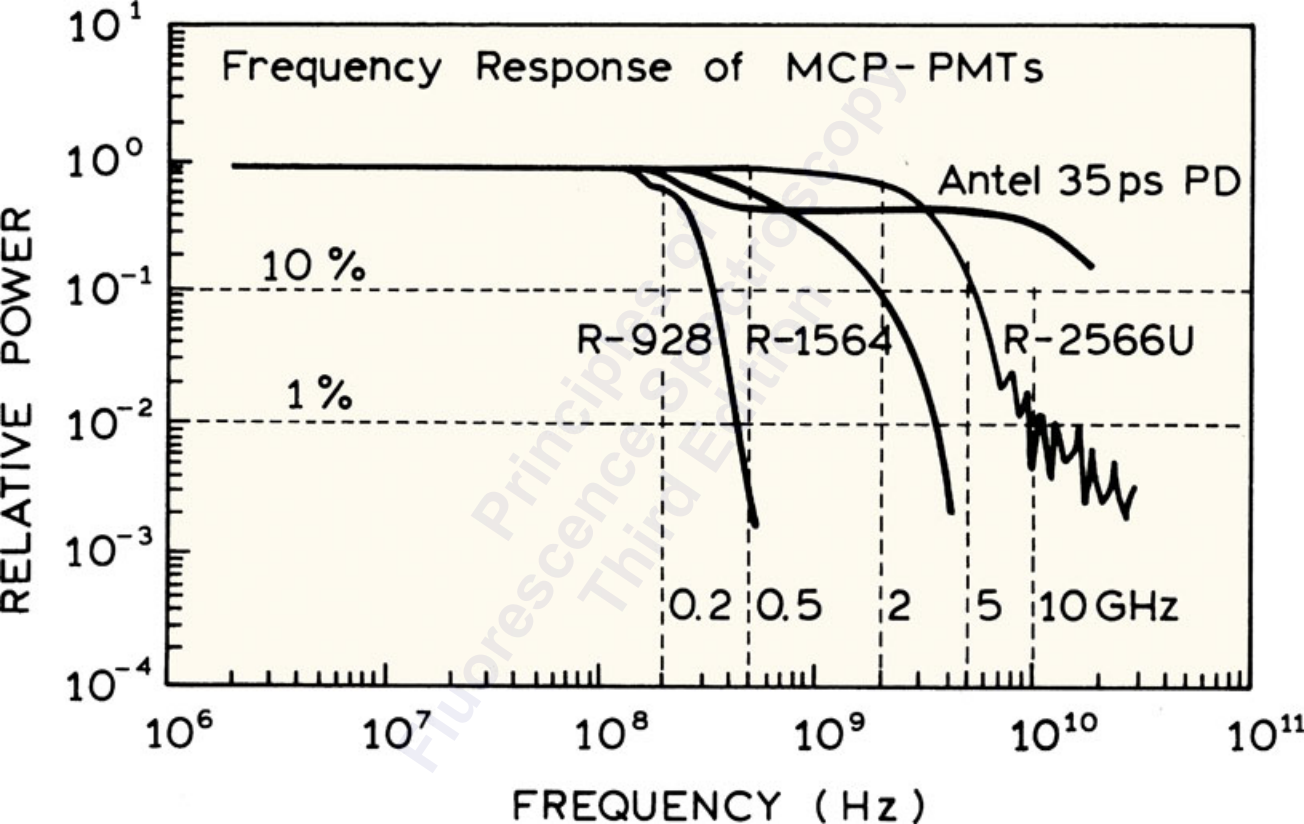
PHASE ANGLE (degrees)  
or MODULATION (%)

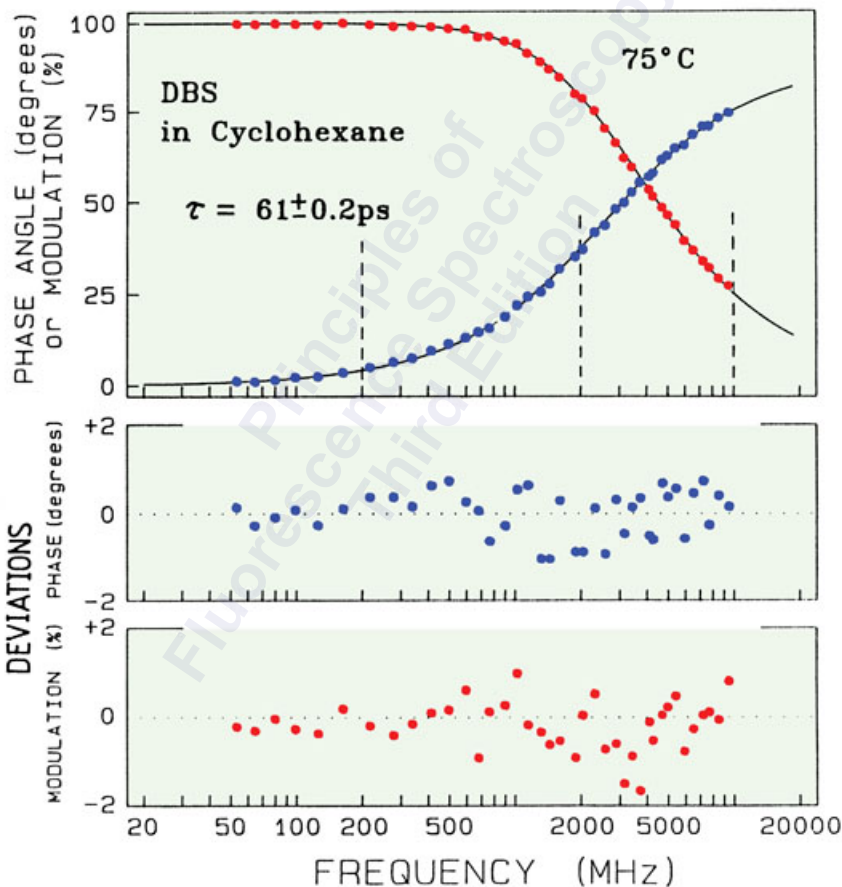
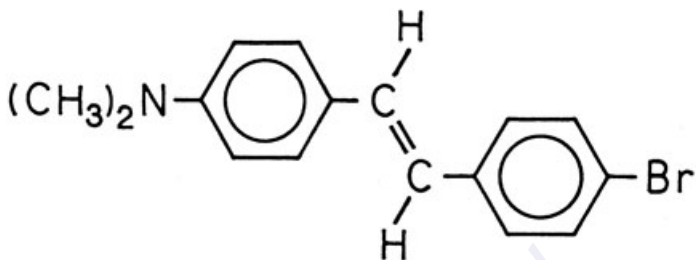




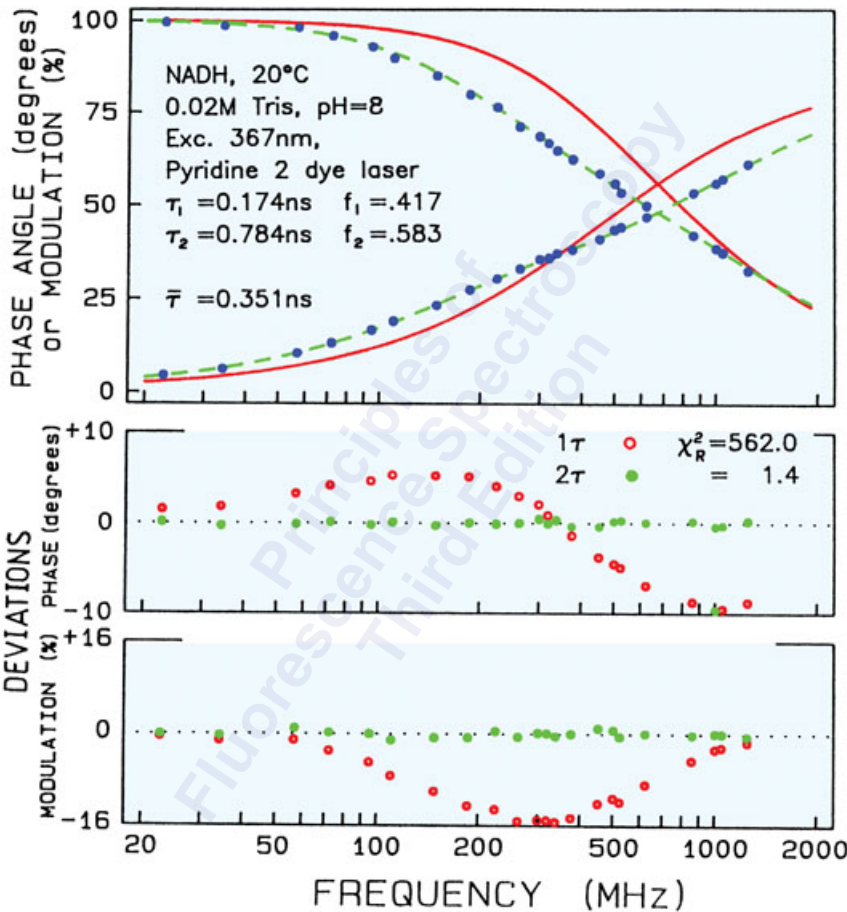
Fourier  
 Transform

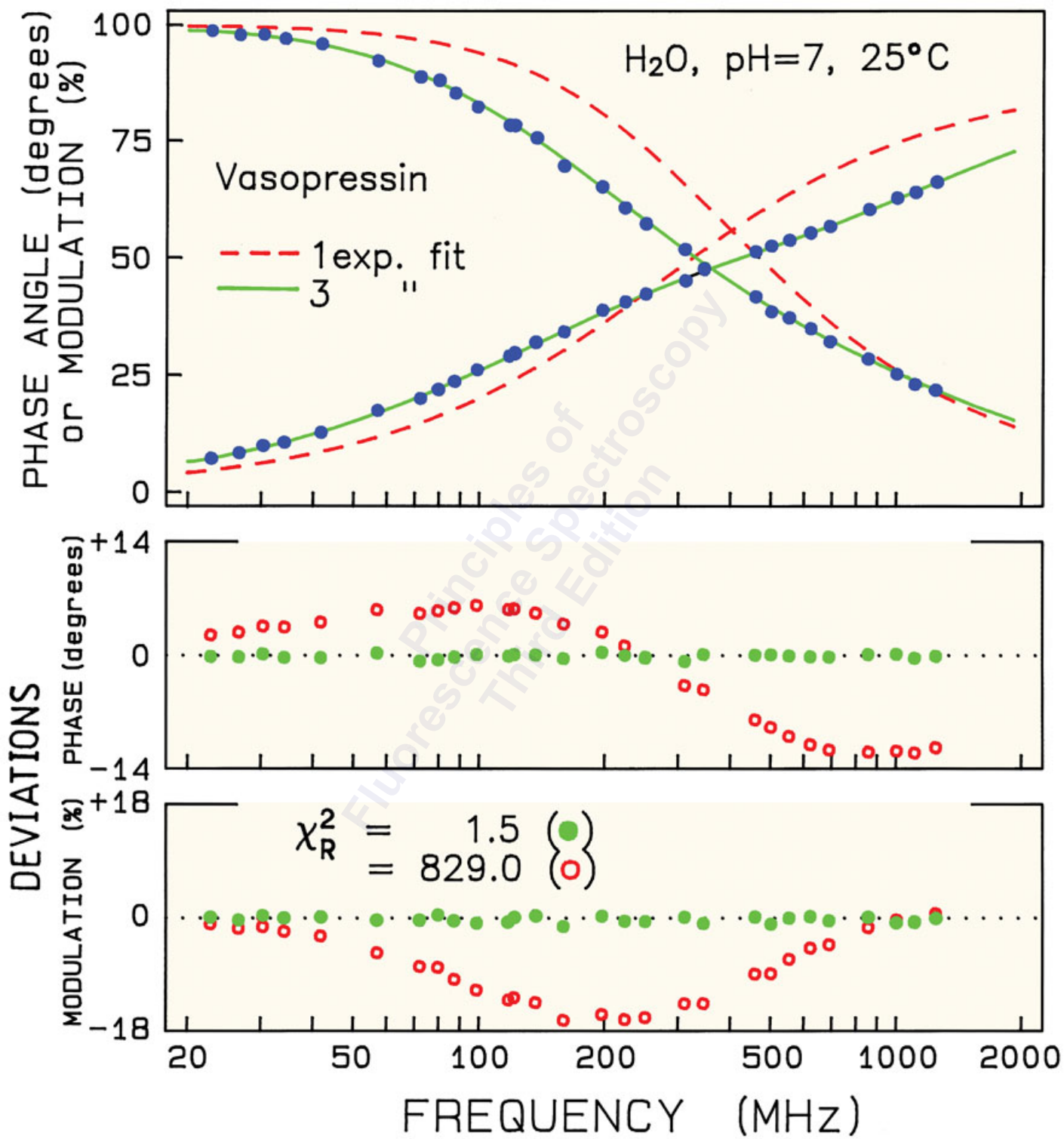


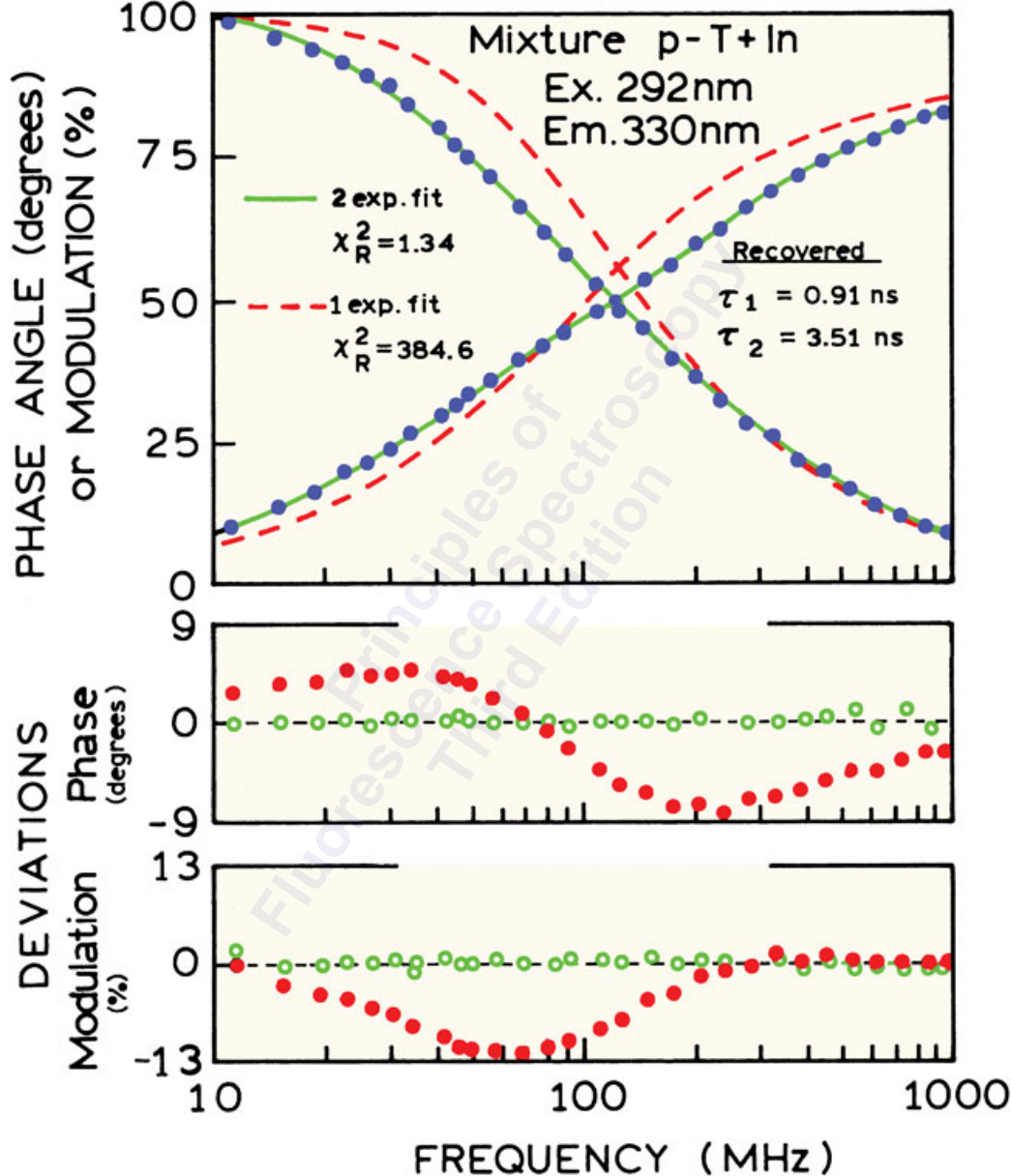


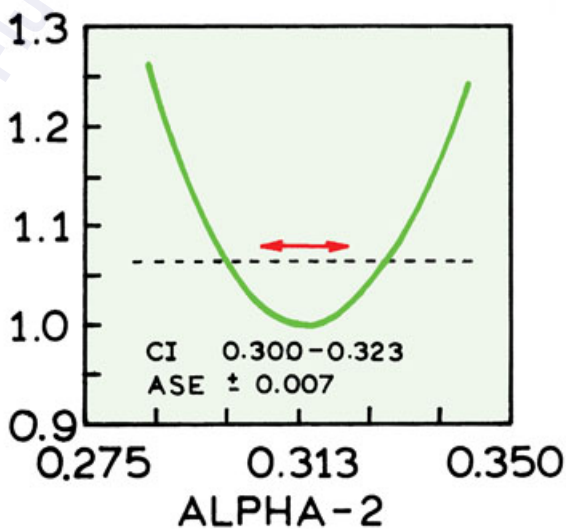
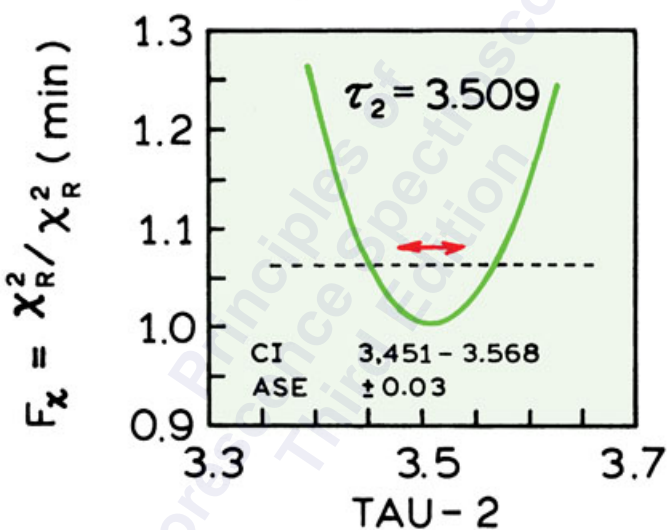
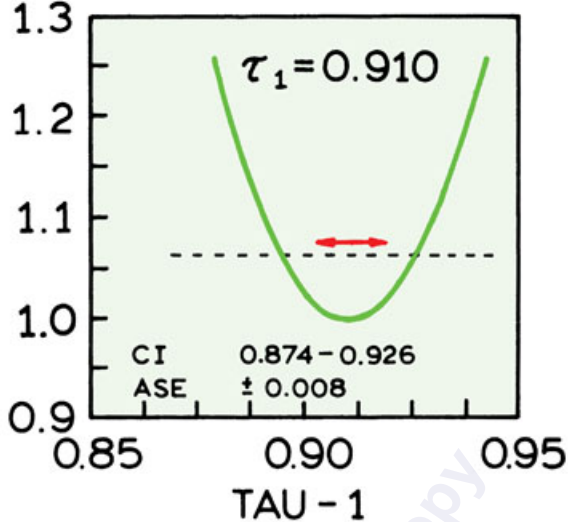


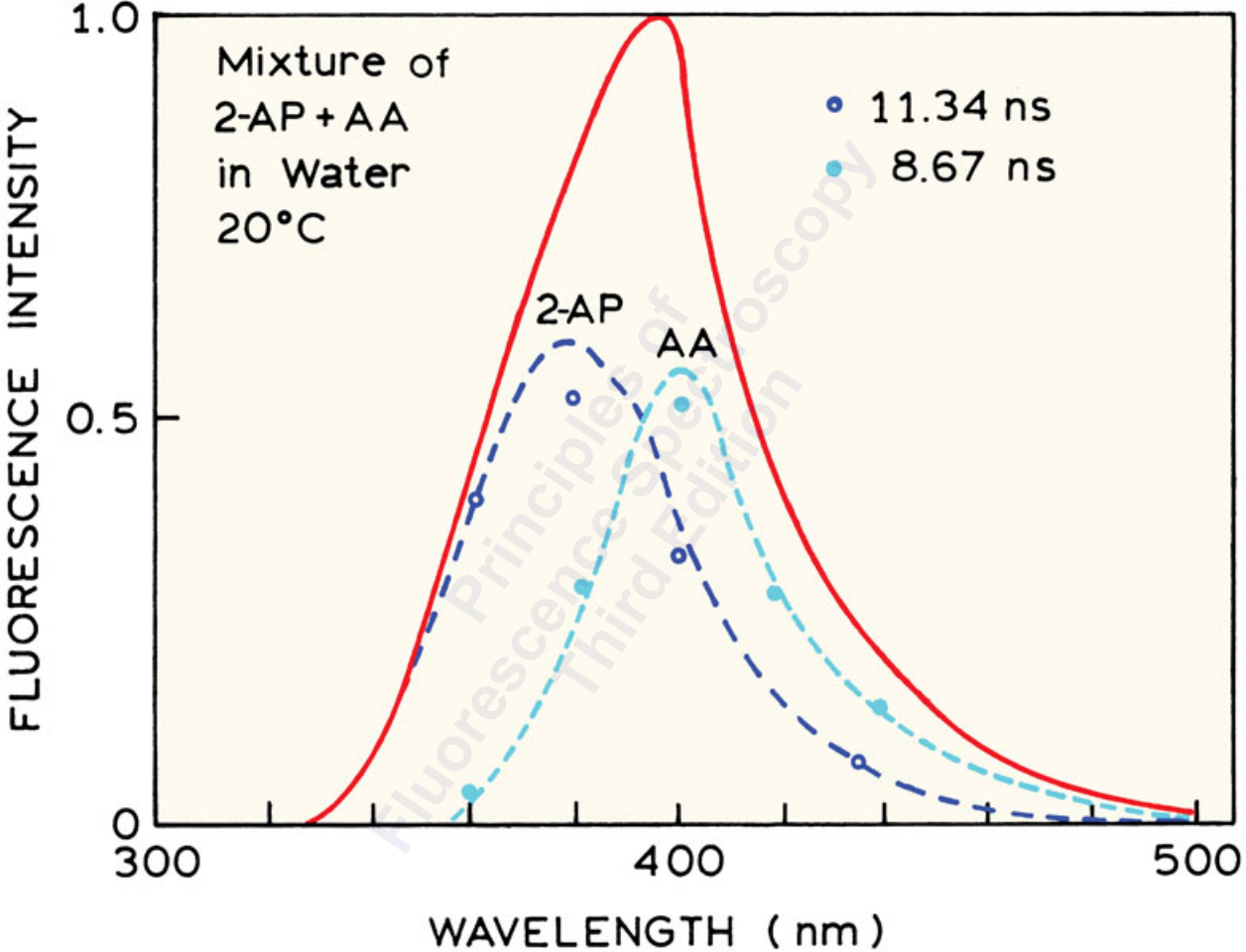


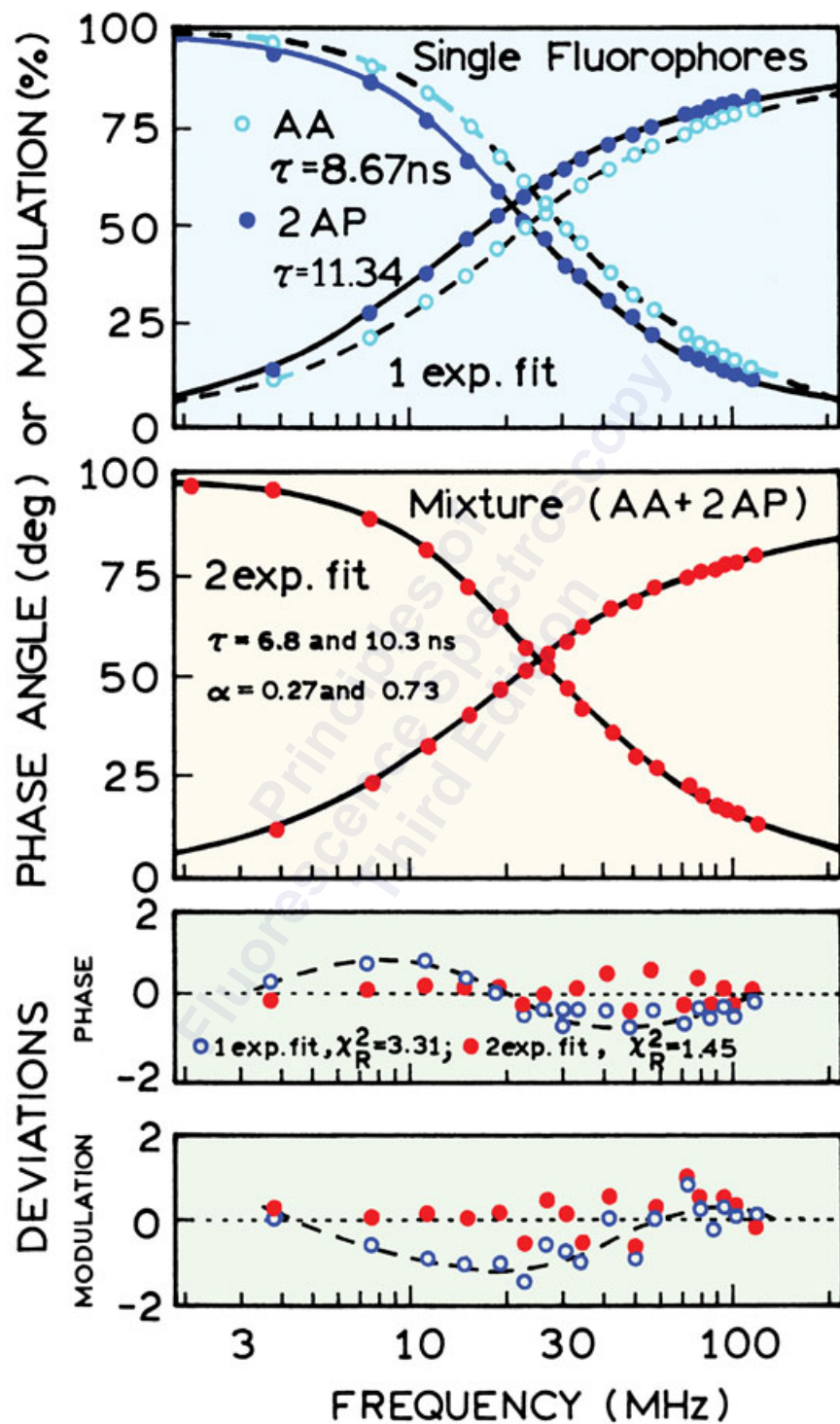




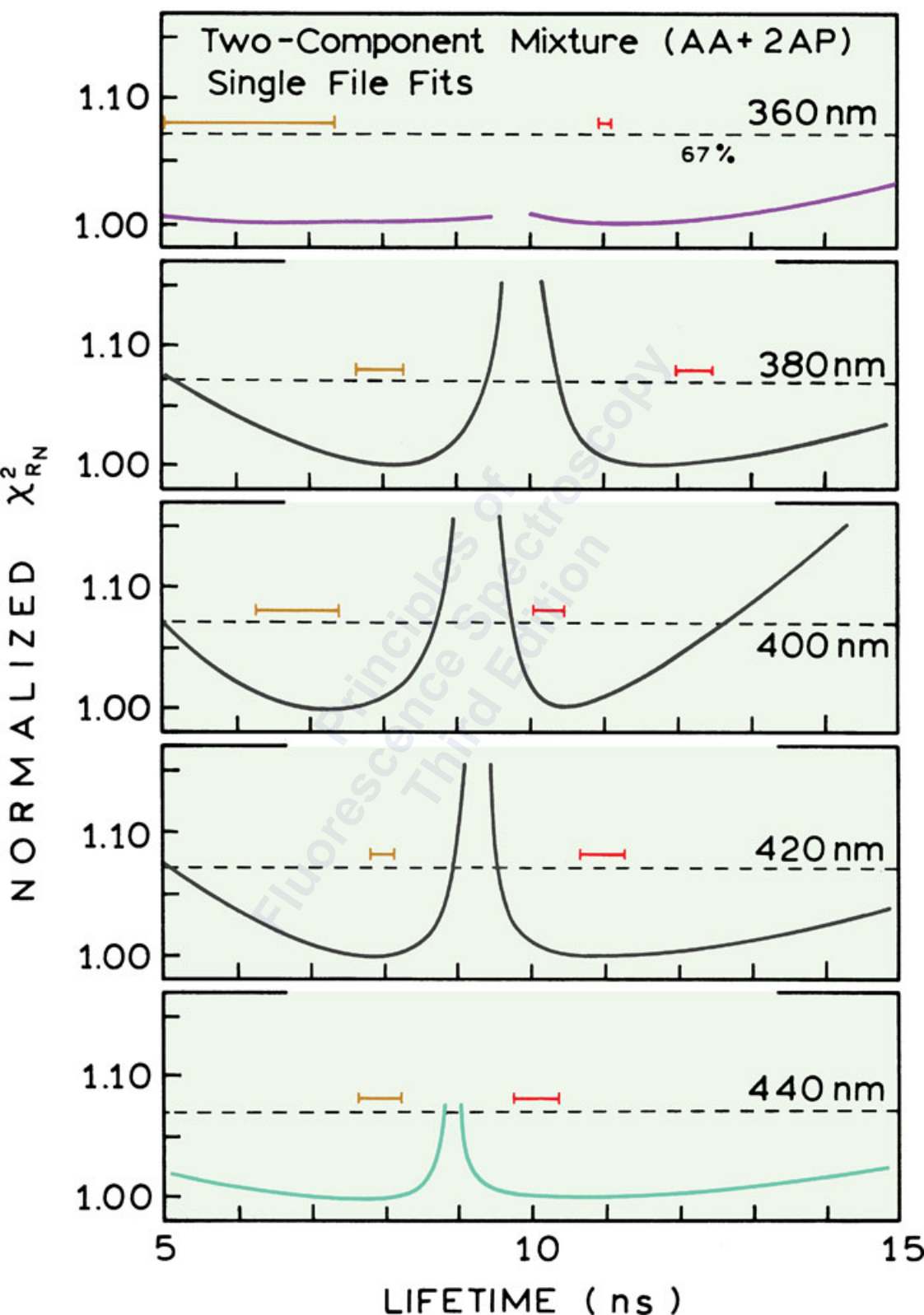


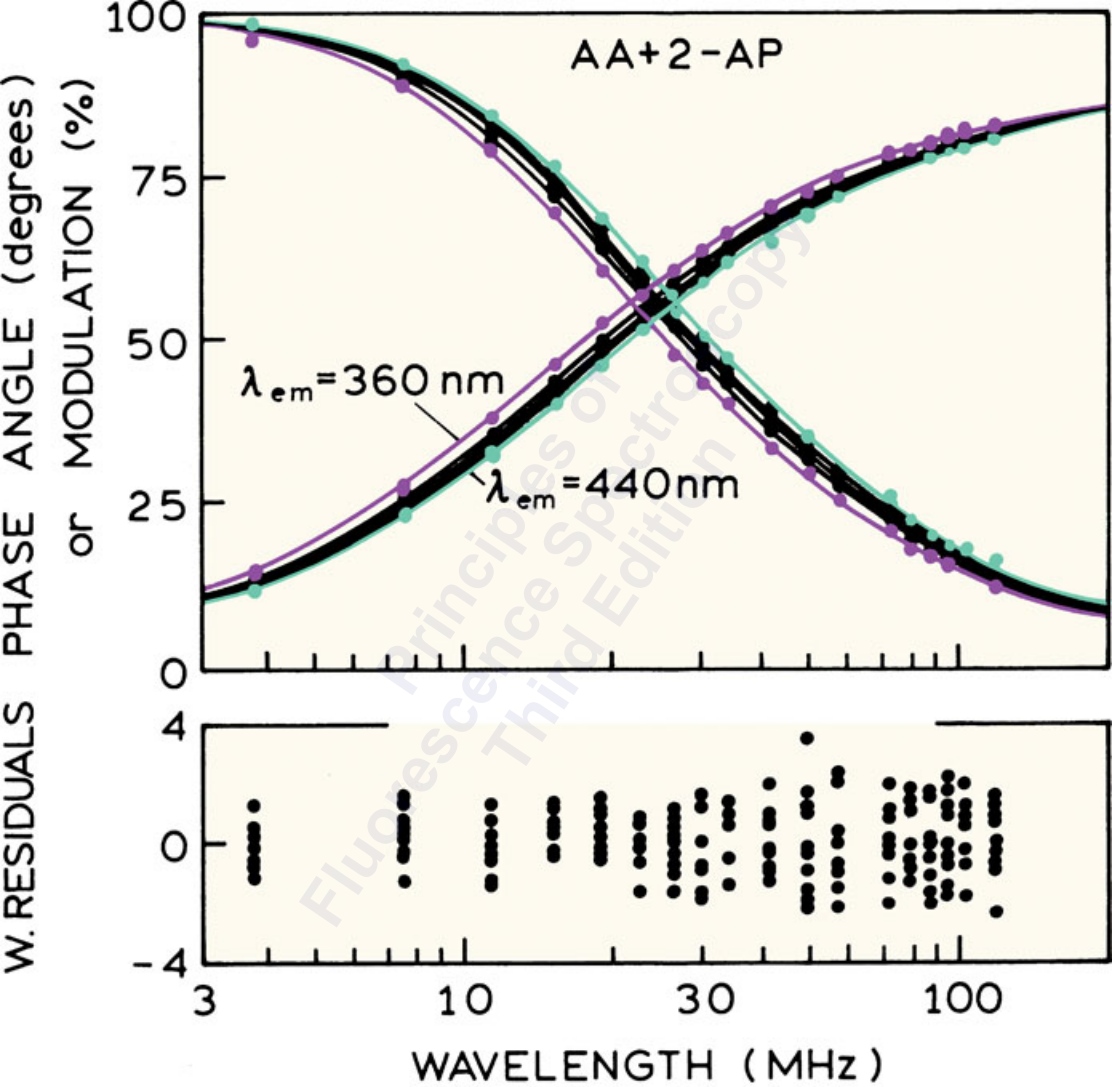




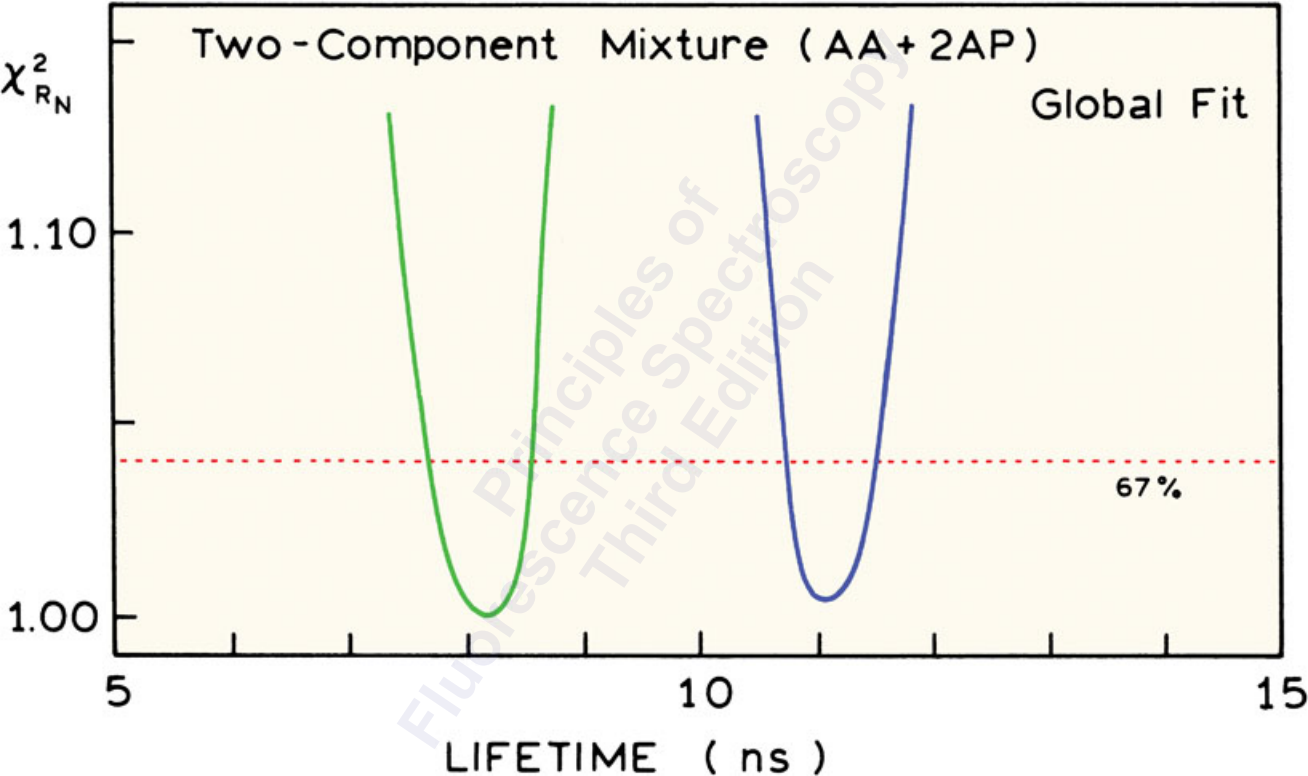


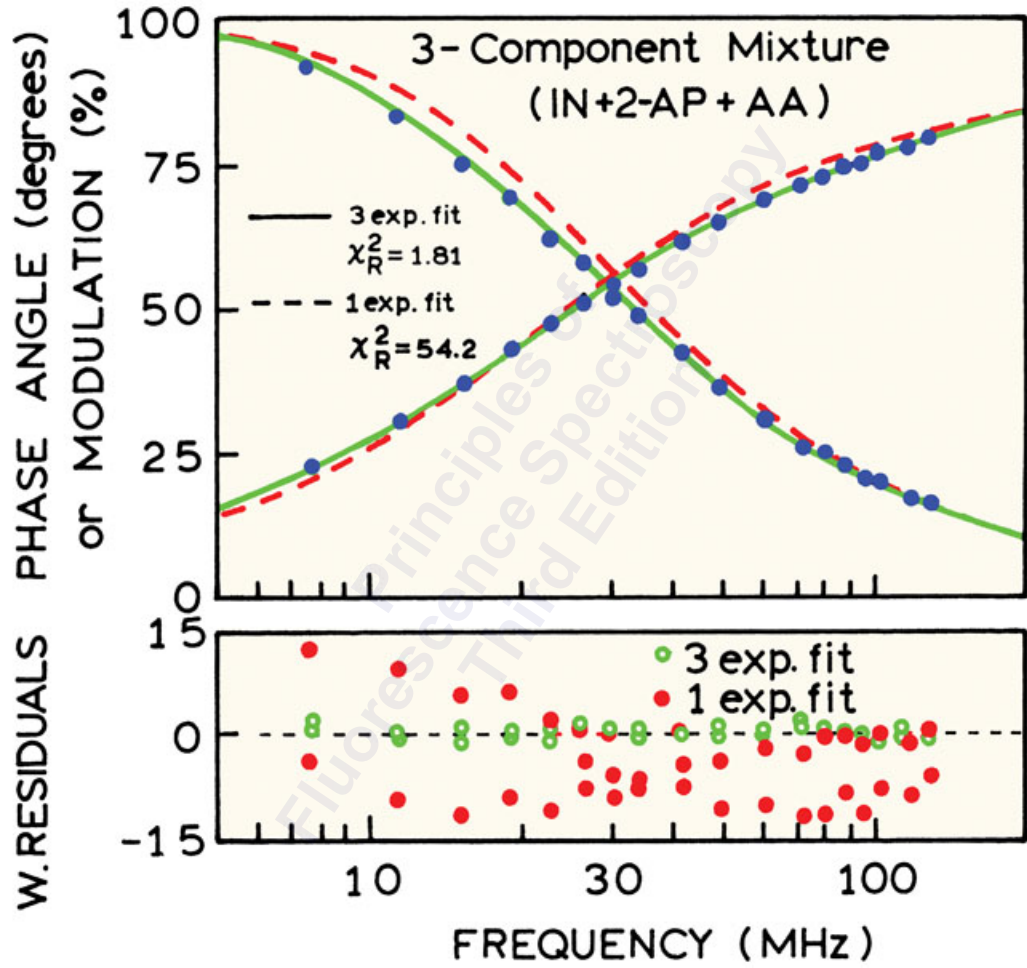












$\chi^2_{RN}$ 

Three Component Mixture of In, AA and 2-AP

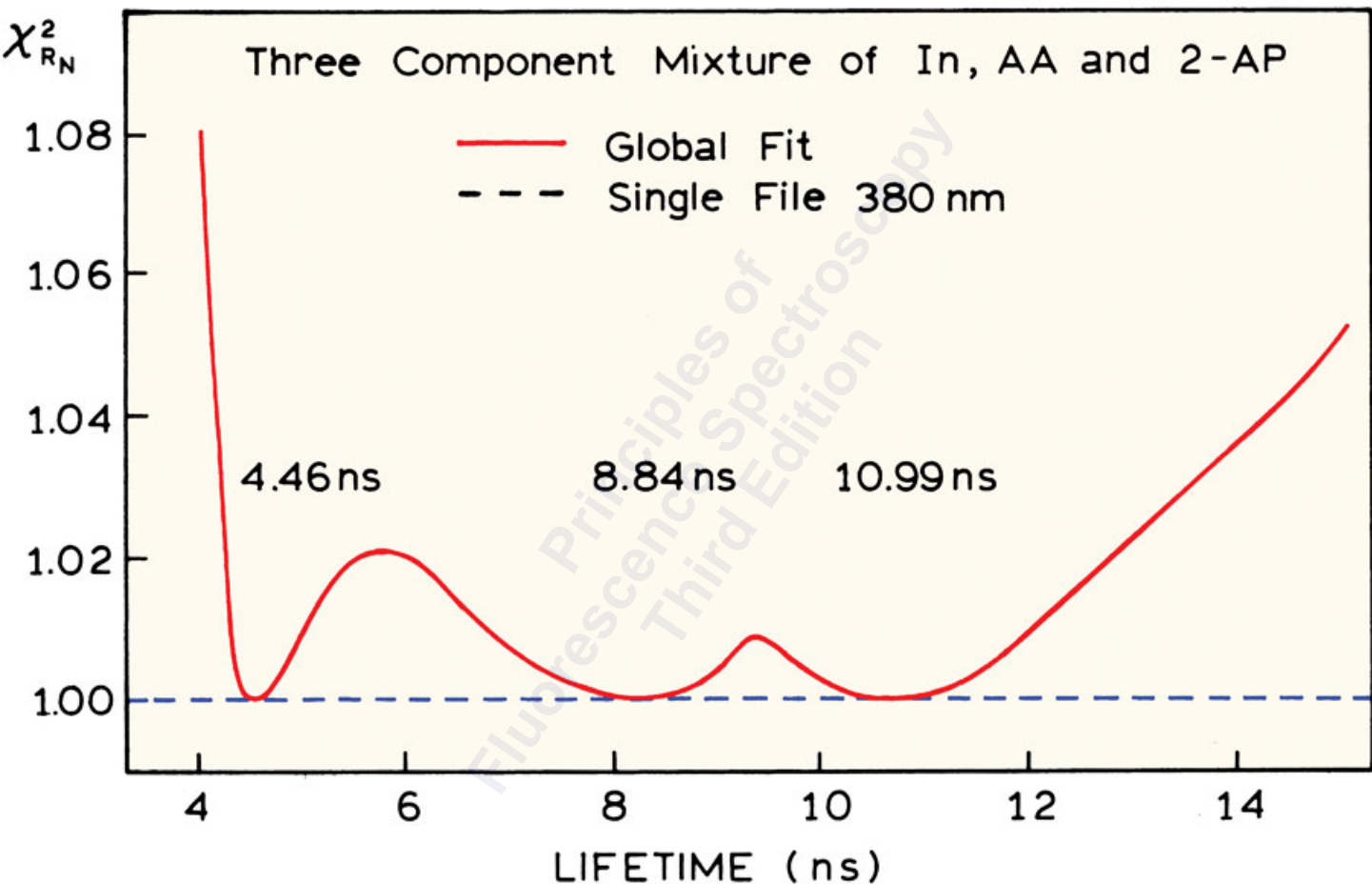
— Global Fit  
- - - Single File 380 nm

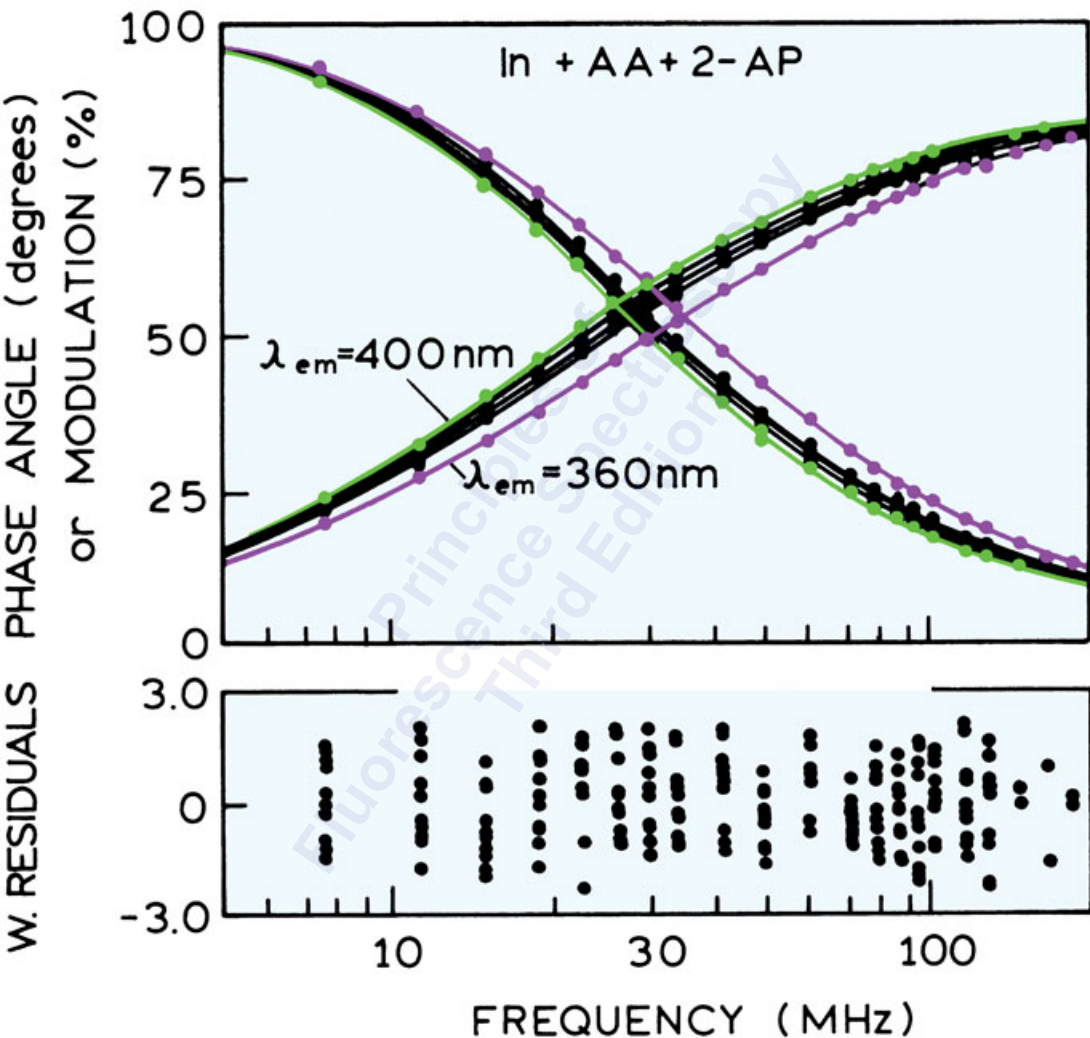
4.46 ns

8.84 ns

10.99 ns

LIFETIME (ns)





FLUORESCENCE INTENSITY

1.0

Three Component Mixture of In, AA and 2-AP  
in Water at 20°C

0.5

Fractional  
Intensities

○ In

□ 2-AP

△ AA

In

2-AP

AA

0

300

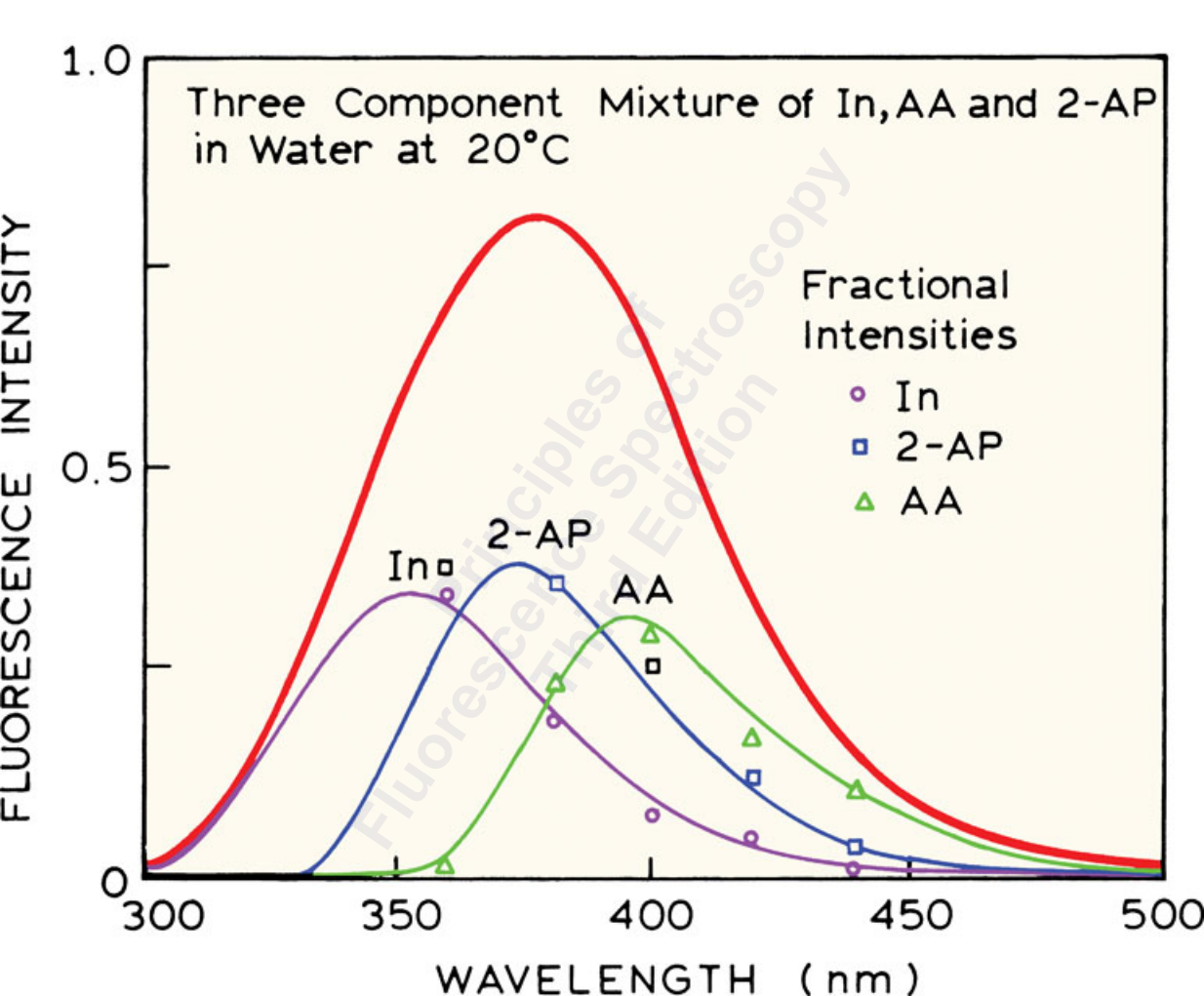
350

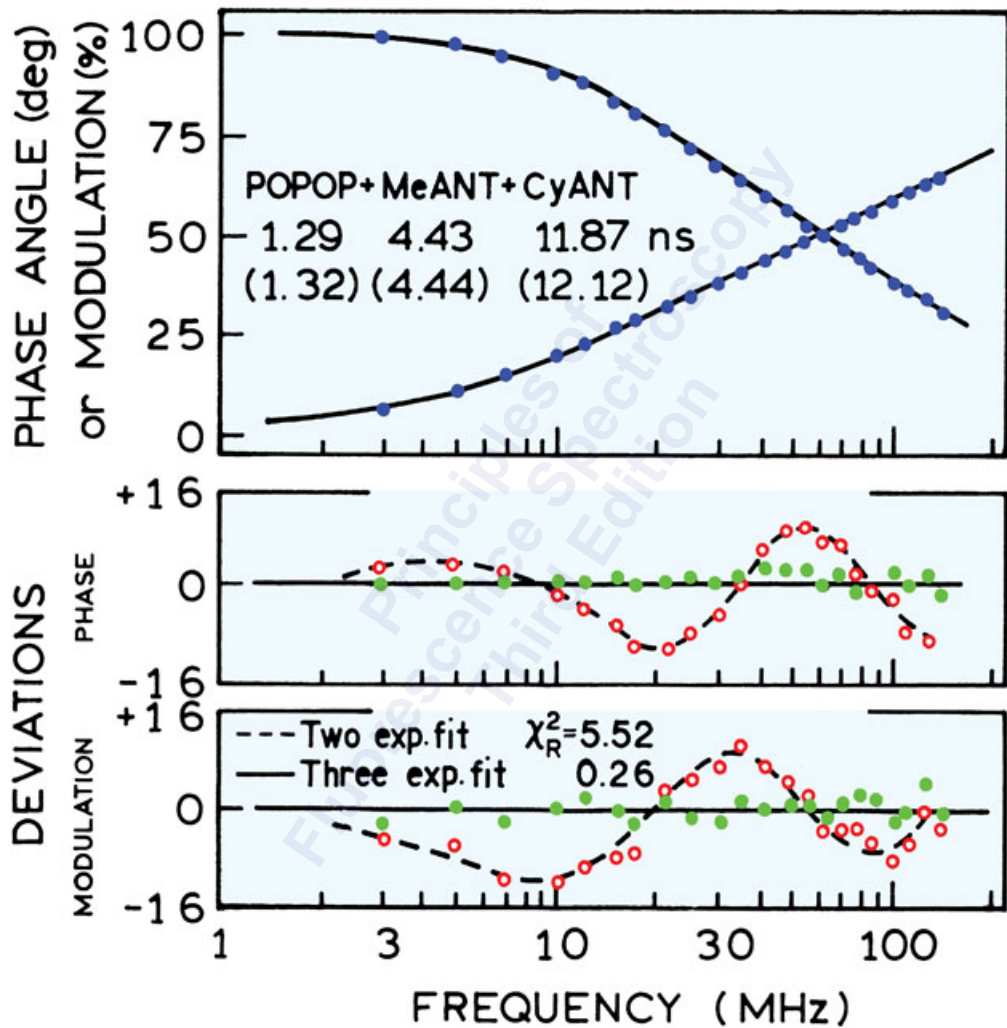
400

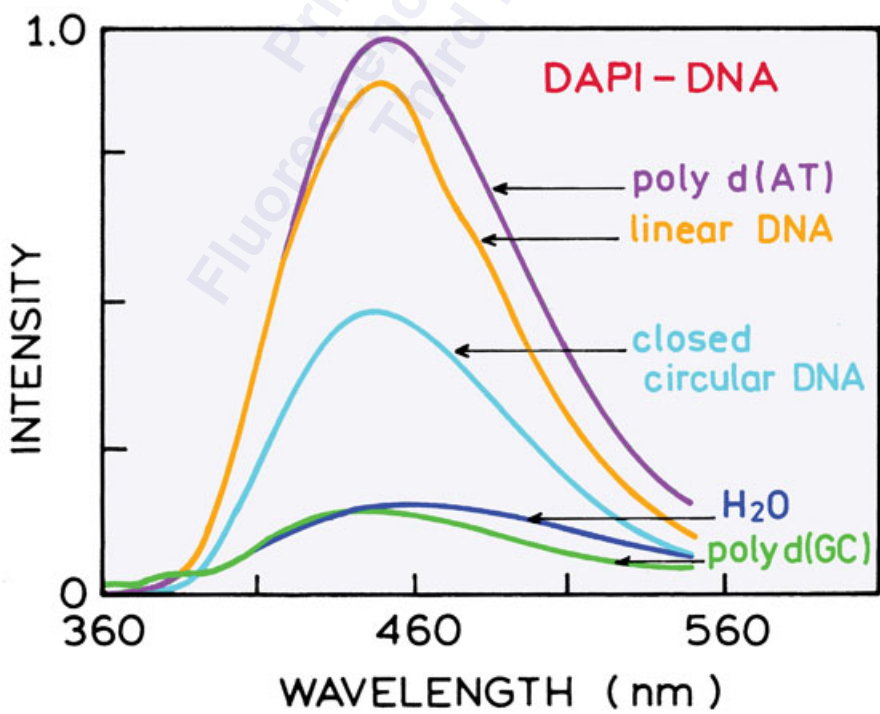
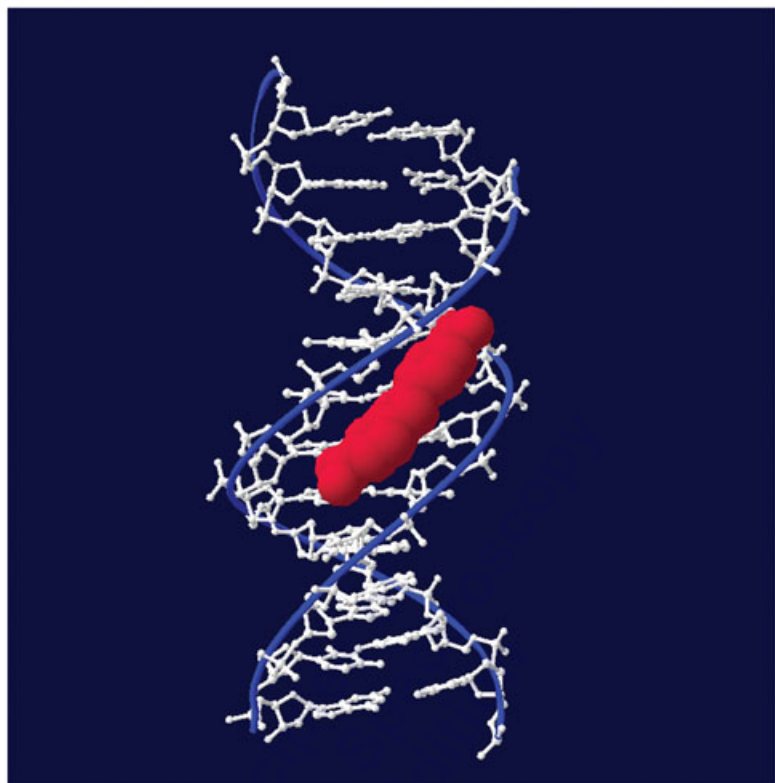
450

500

WAVELENGTH (nm)

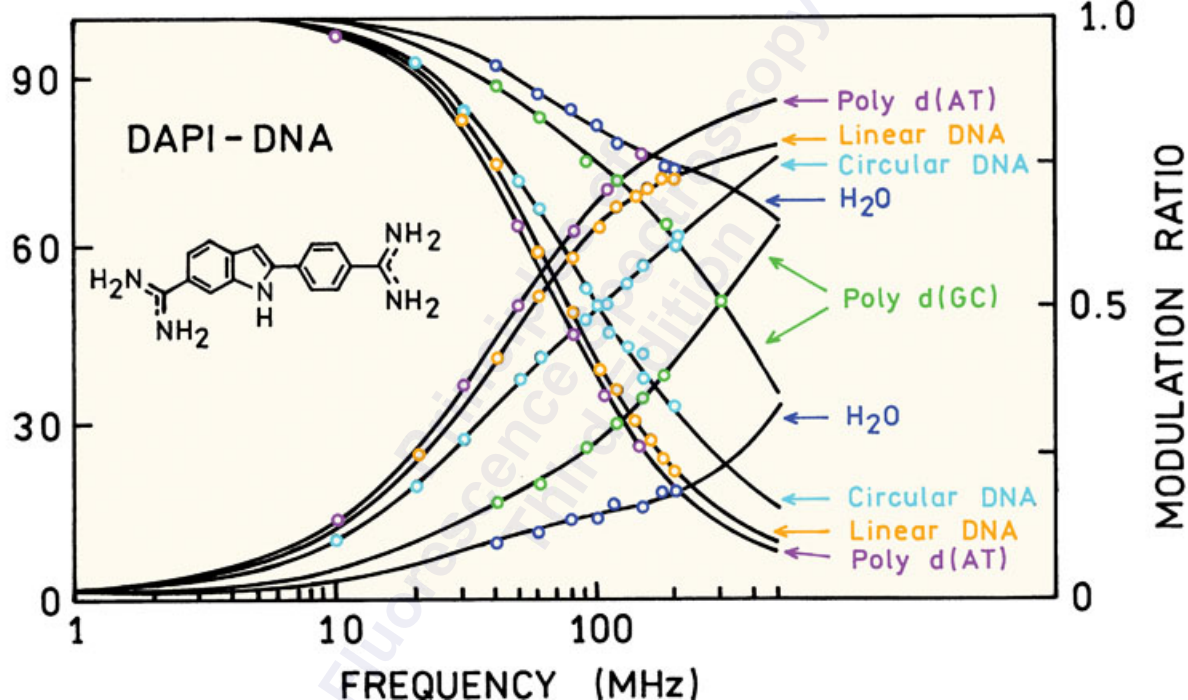




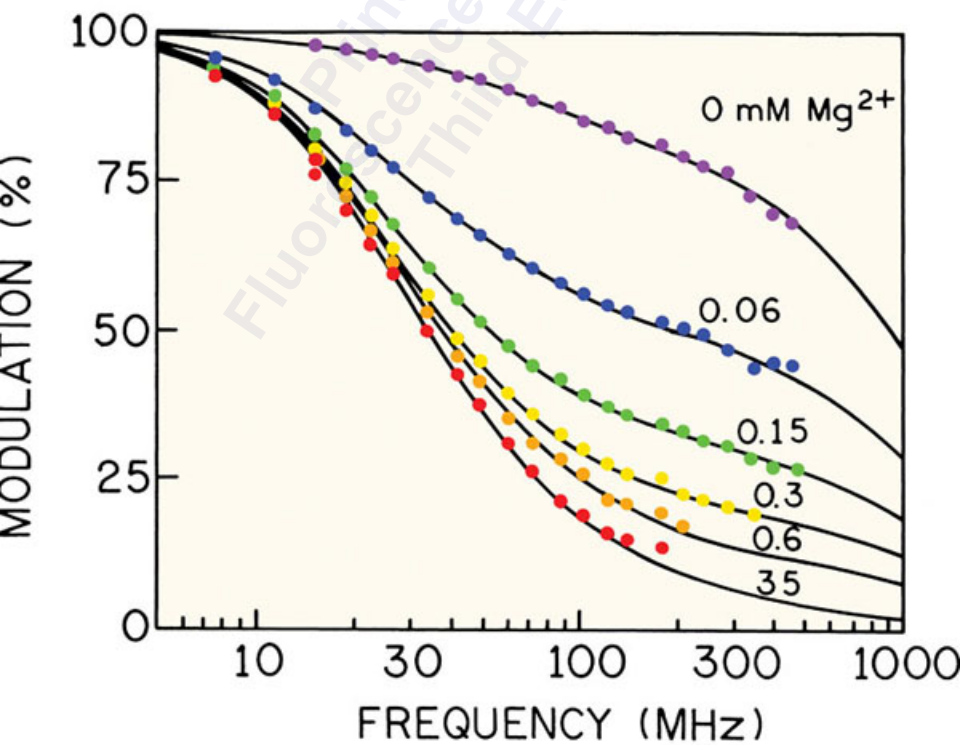
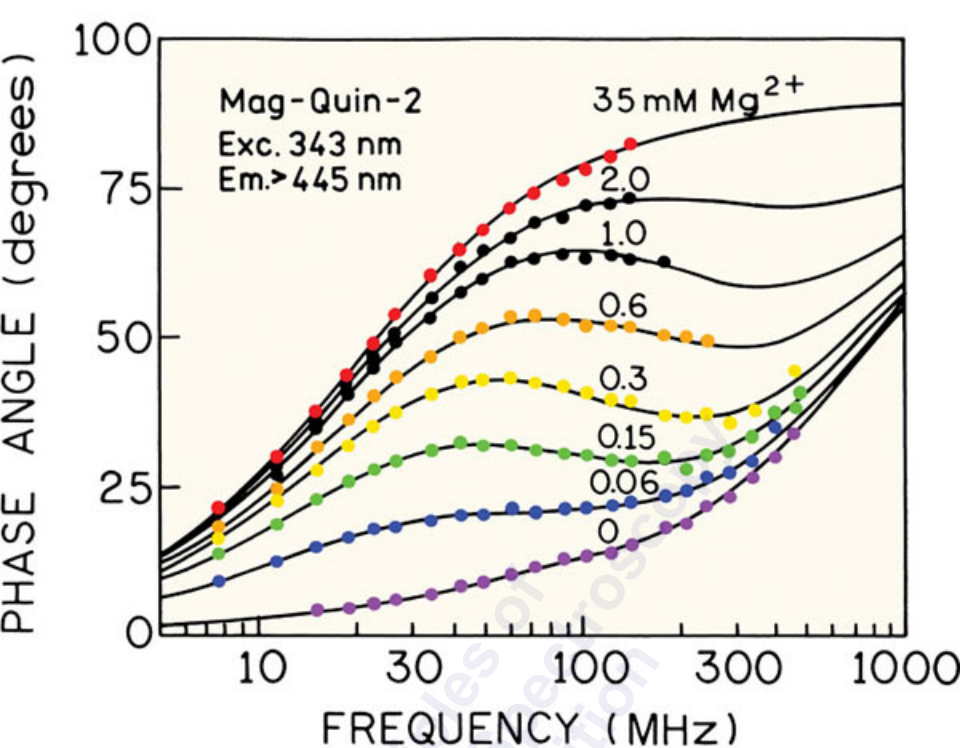


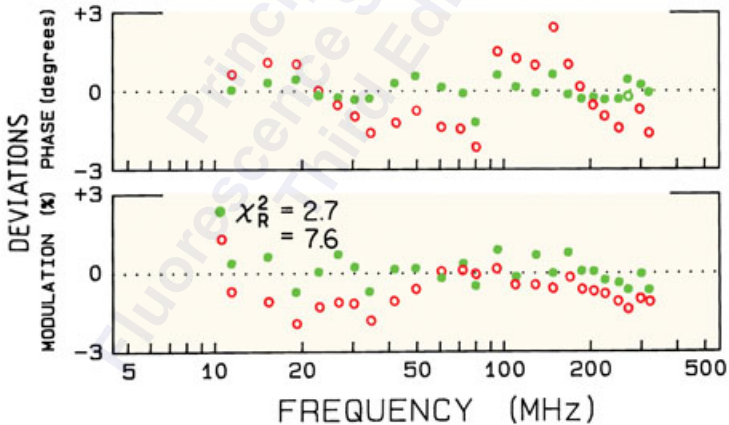
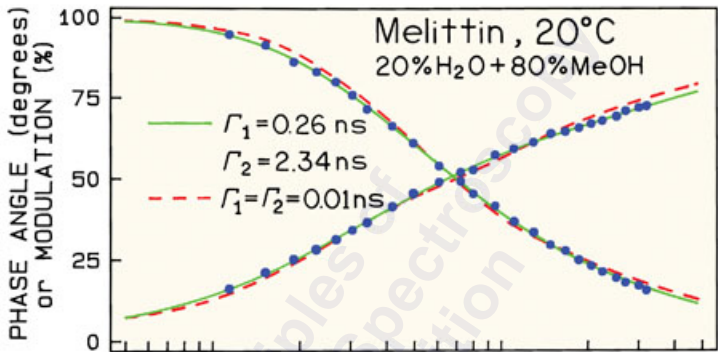


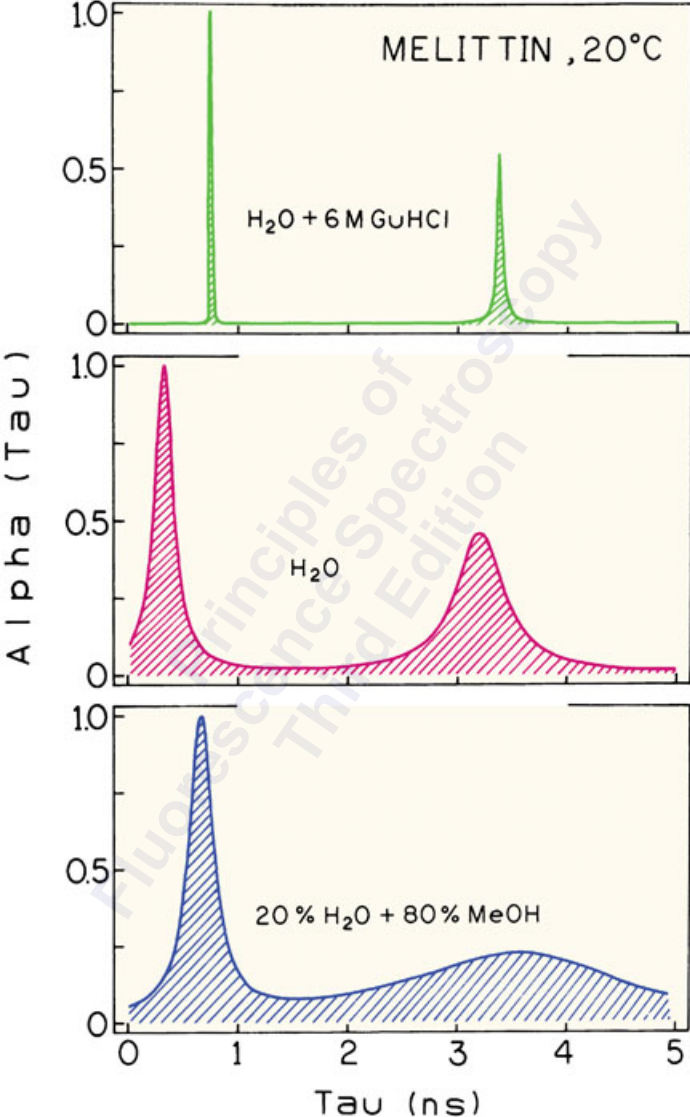
PHASE (degrees)

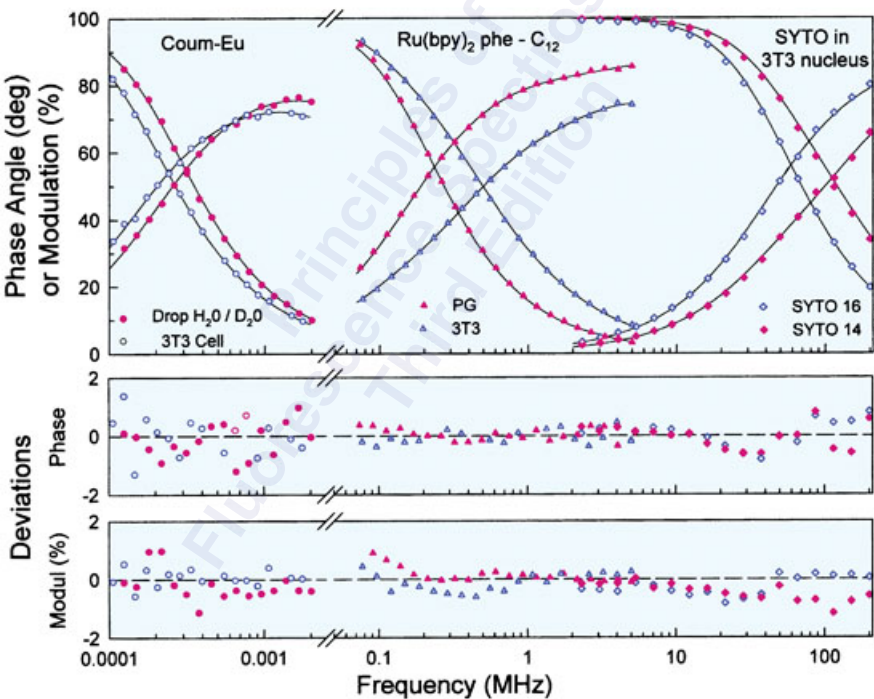
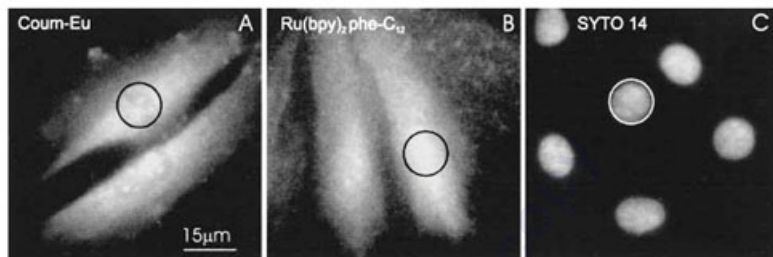


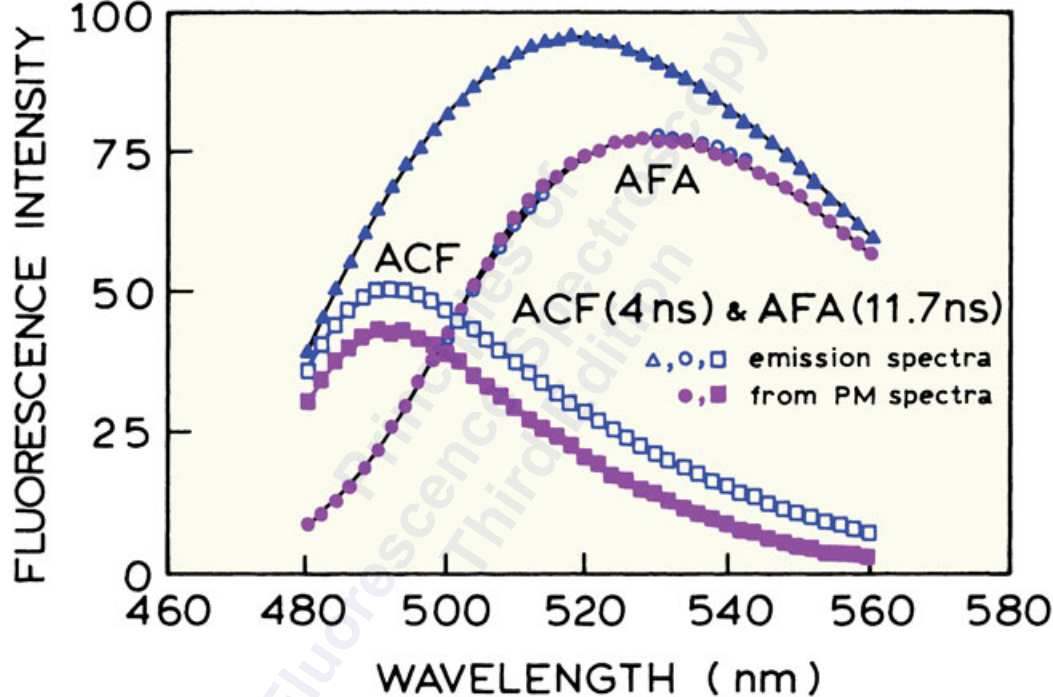


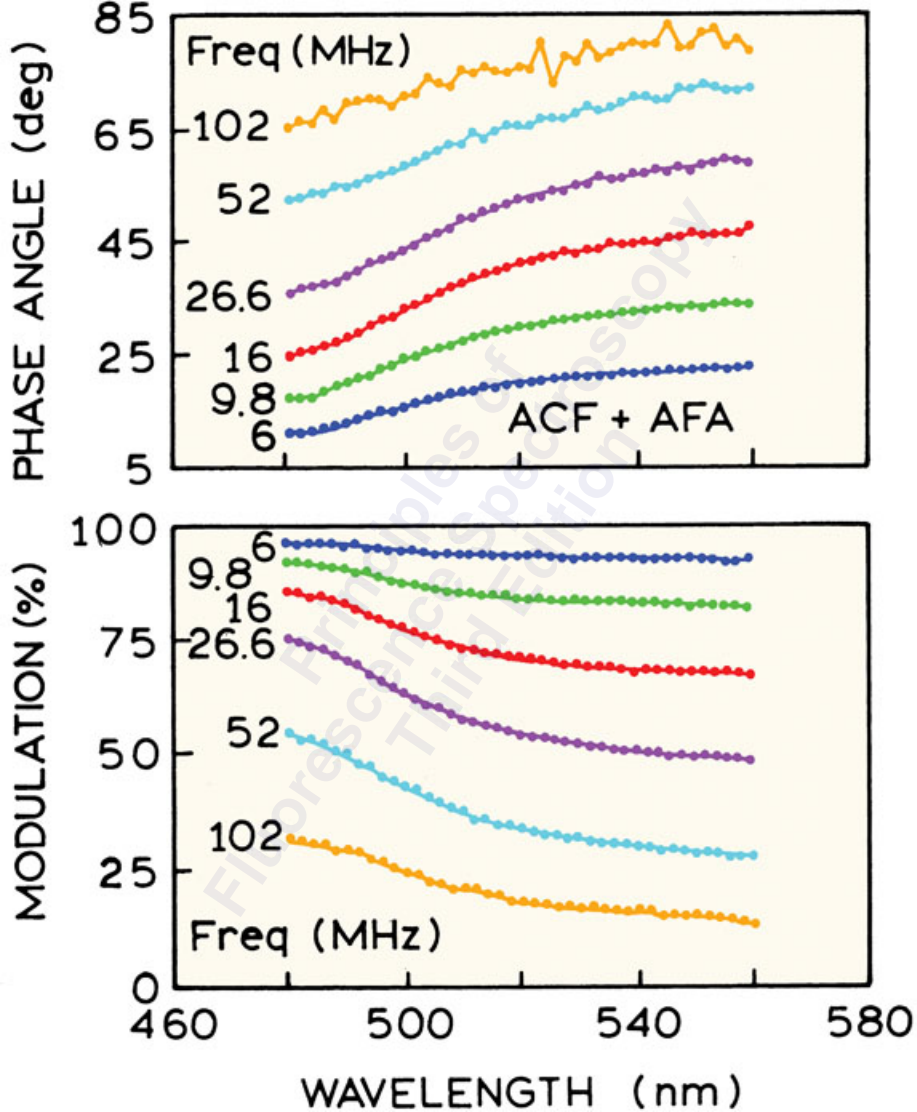


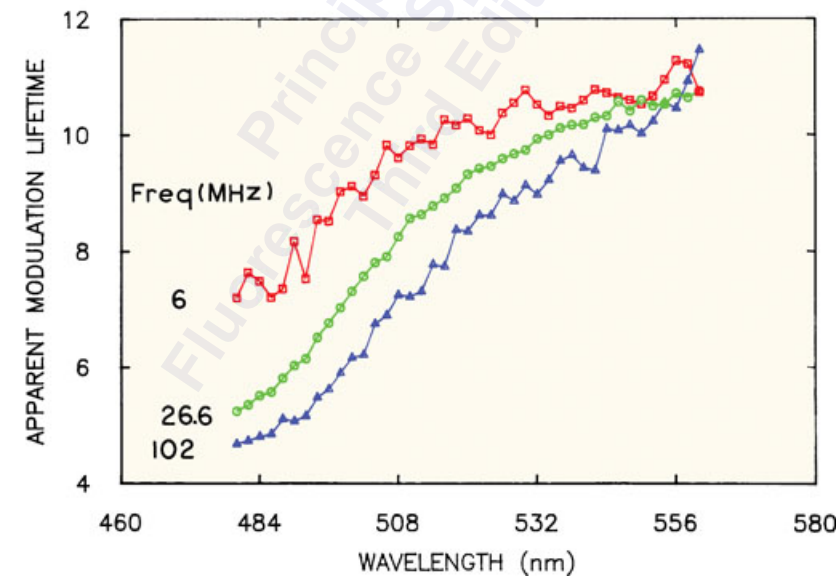
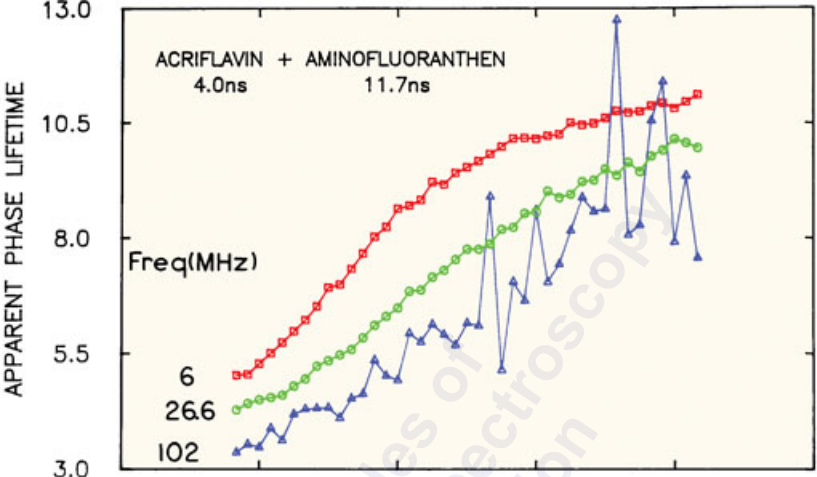












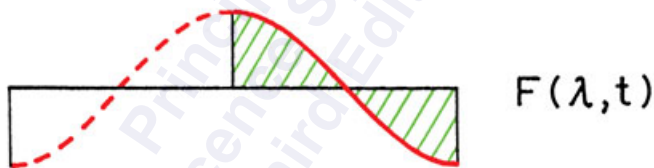


$$\phi_D = 0$$

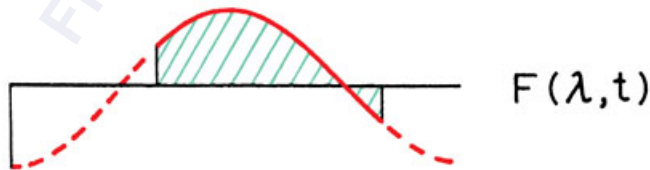

 $P(t)$ 

 $F(\lambda, t)$ 

$$\phi_D = \phi + 90^\circ$$


 $P(t)$ 

 $F(\lambda, t)$ 

$\phi_D$  between  $\phi$  and  $\phi + 90^\circ$

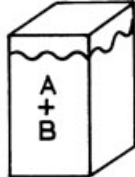

 $P(t)$ 

 $F(\lambda, t)$



Excitation



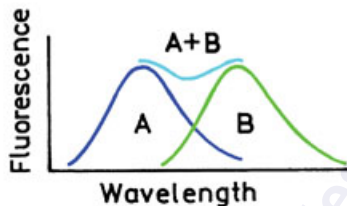
$$i(t) = I + m \cos \omega t$$



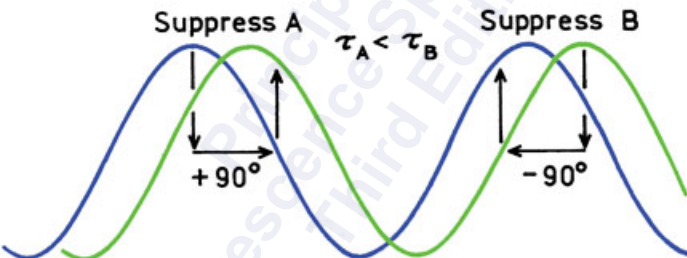
Emission



$$F(t) = I + m' \cos(\omega t - \phi)$$

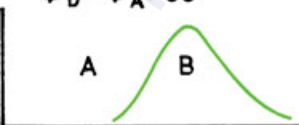


$$I = k I_0 \cos(\phi_D - \phi_i)$$

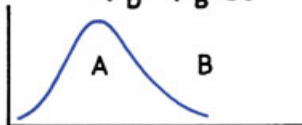


Phase Sensitive Fluorescence Spectra

$$\phi_D = \phi_A + 90^\circ$$



$$\phi_D = \phi_B - 90^\circ$$



Wavelength

Wavelength

FLUORESCENCE INTENSITY

Quinine sulfate  
Steady state

Phase  
sensitive

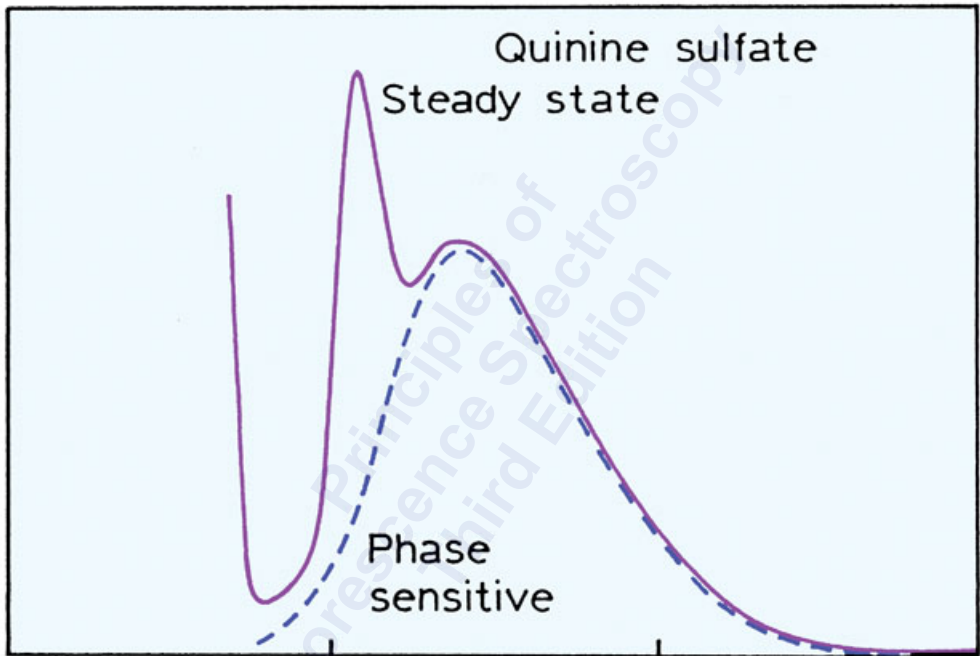
300

400

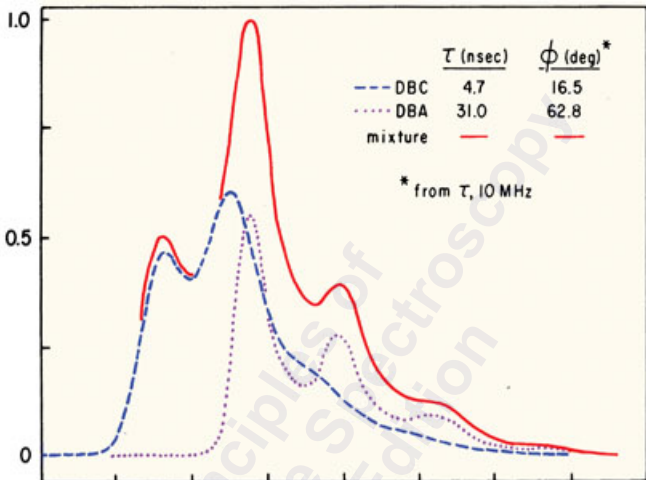
500

600

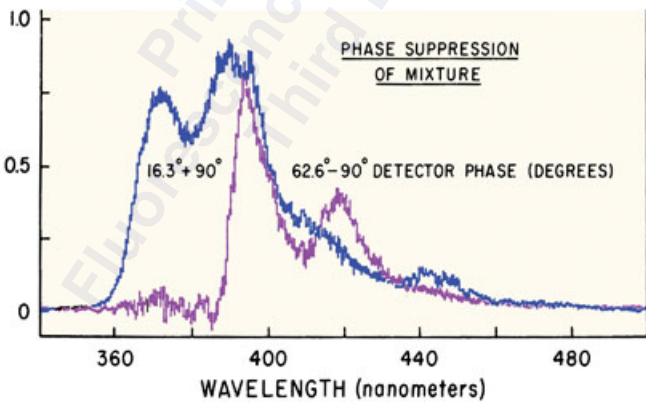
WAVELENGTH (nm)

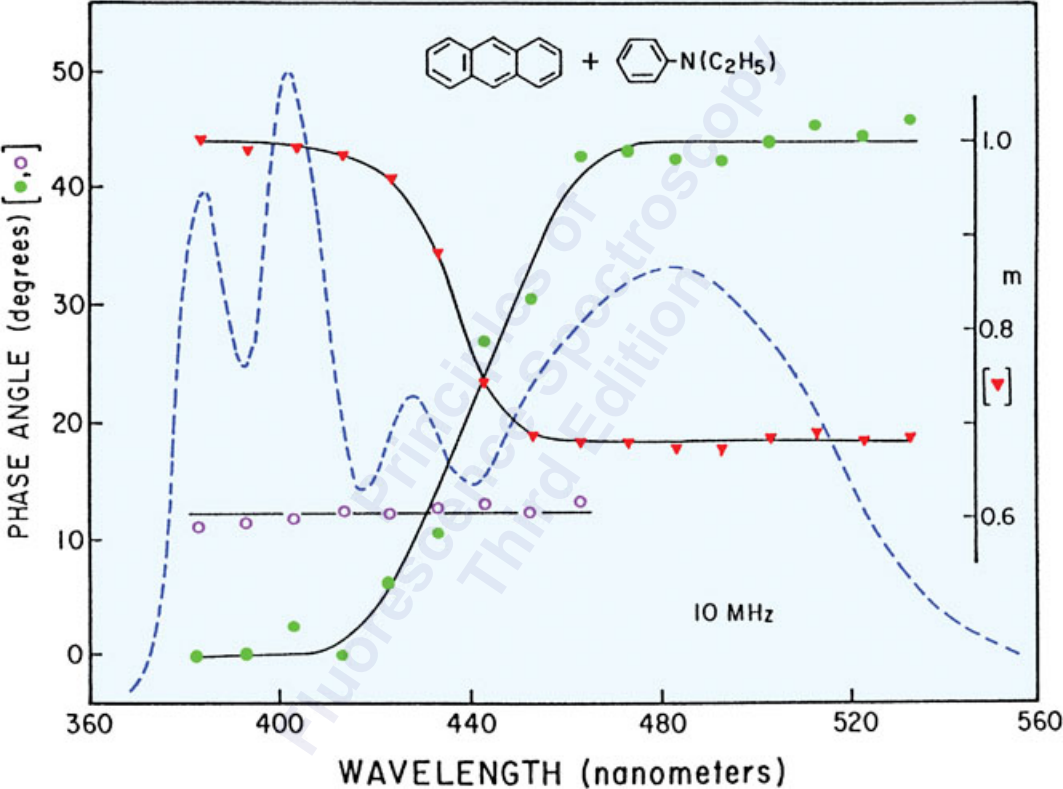


STEADY STATE FLUORESCENCE

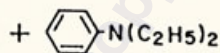
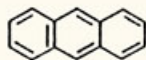


PHASE SENSITIVE FLUORESCENCE



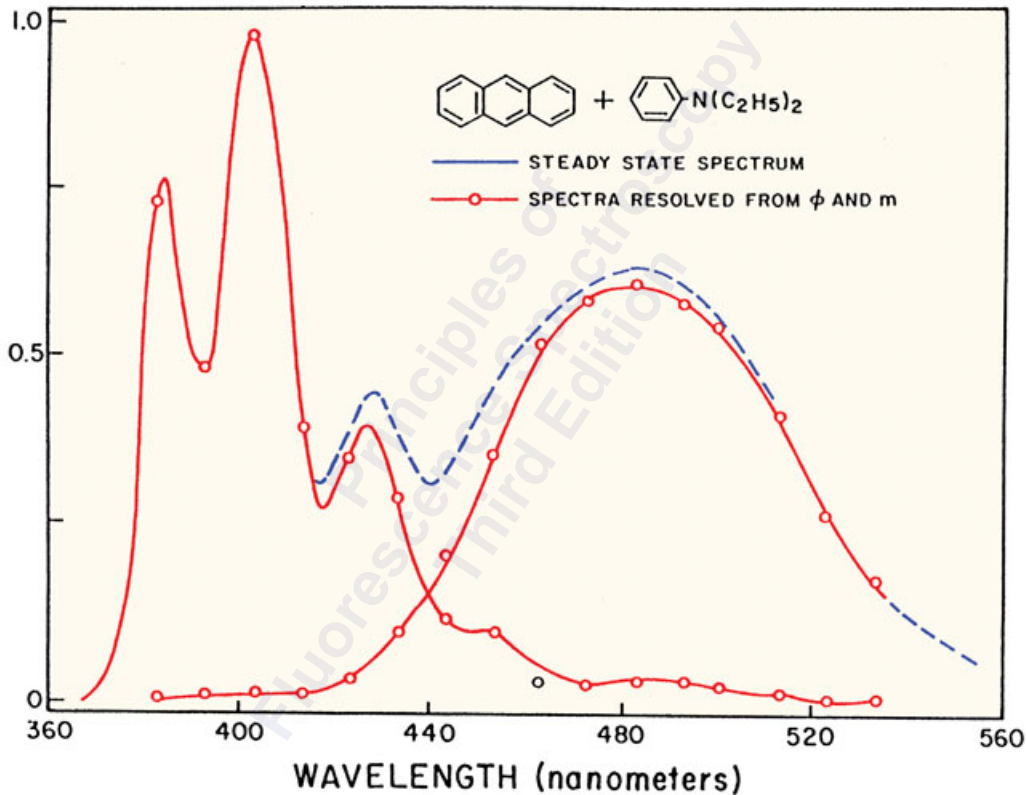


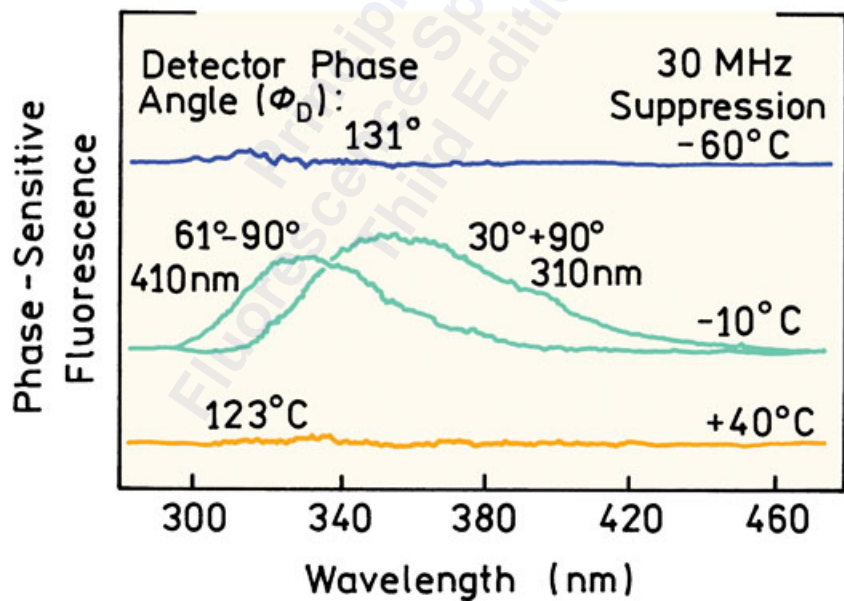
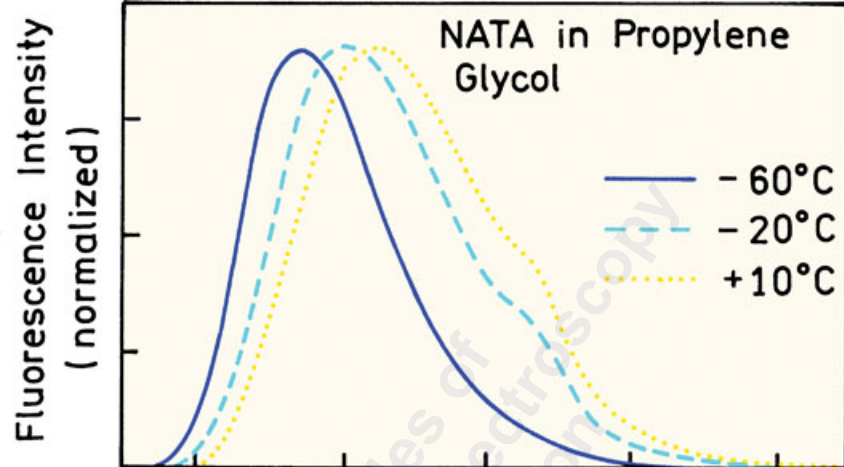
FRACTIONAL FLUORESCENCE INTENSITY

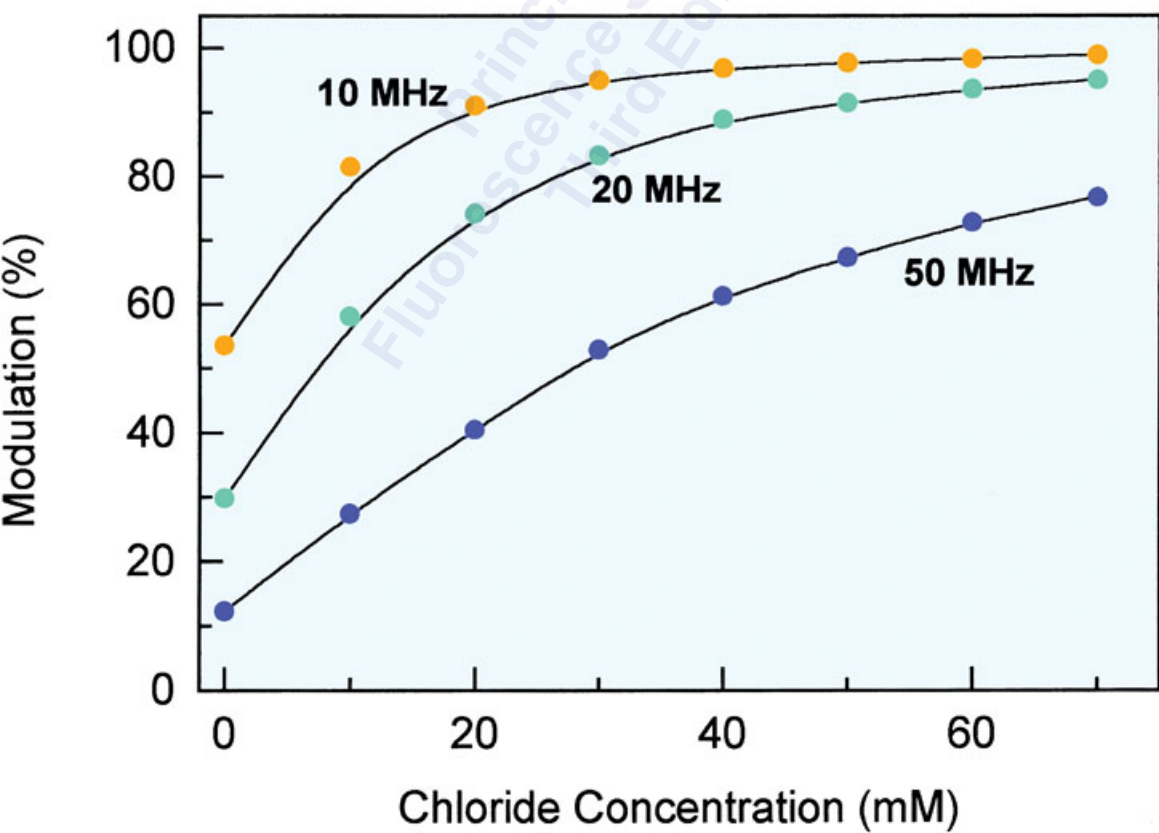
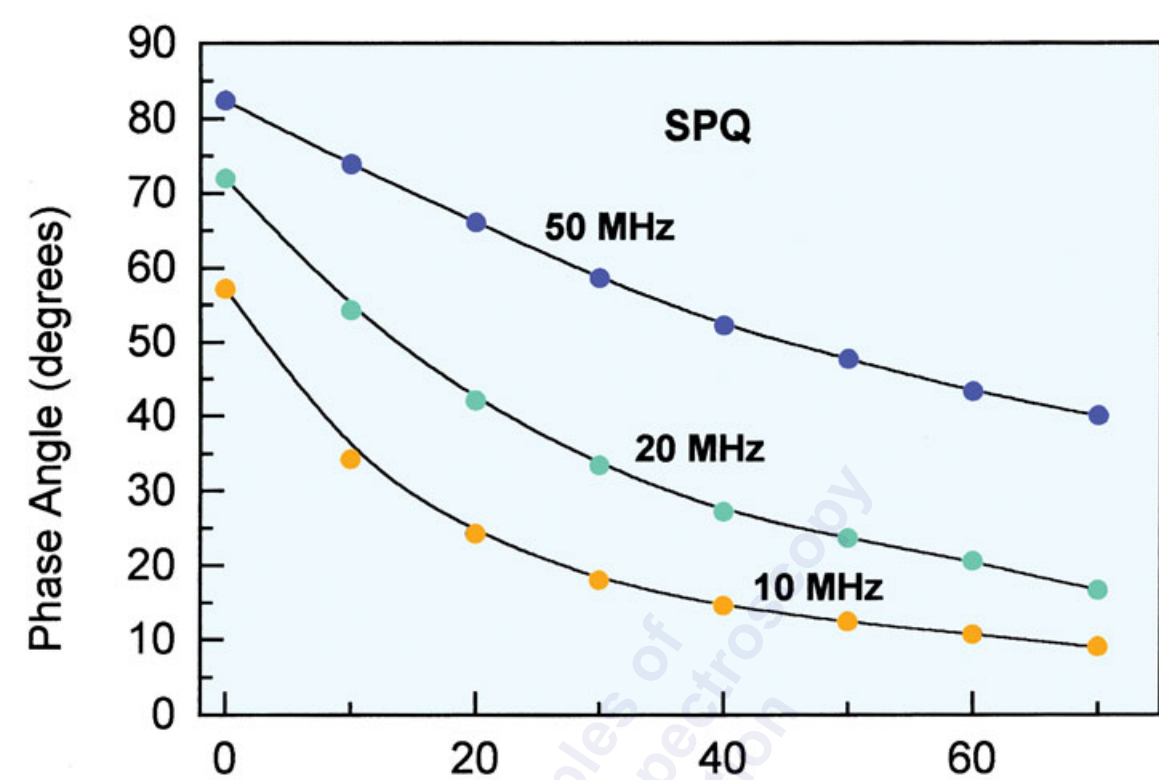


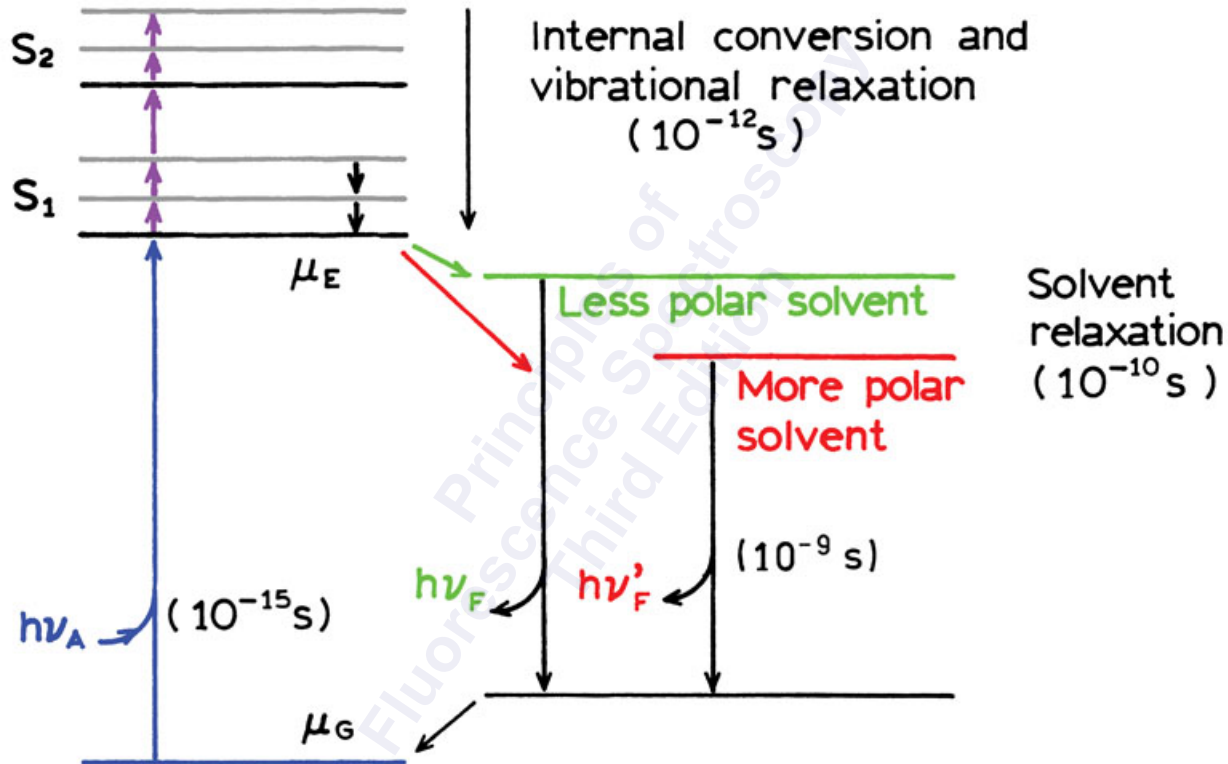
— STEADY STATE SPECTRUM

—○— SPECTRA RESOLVED FROM  $\phi$  AND  $m$

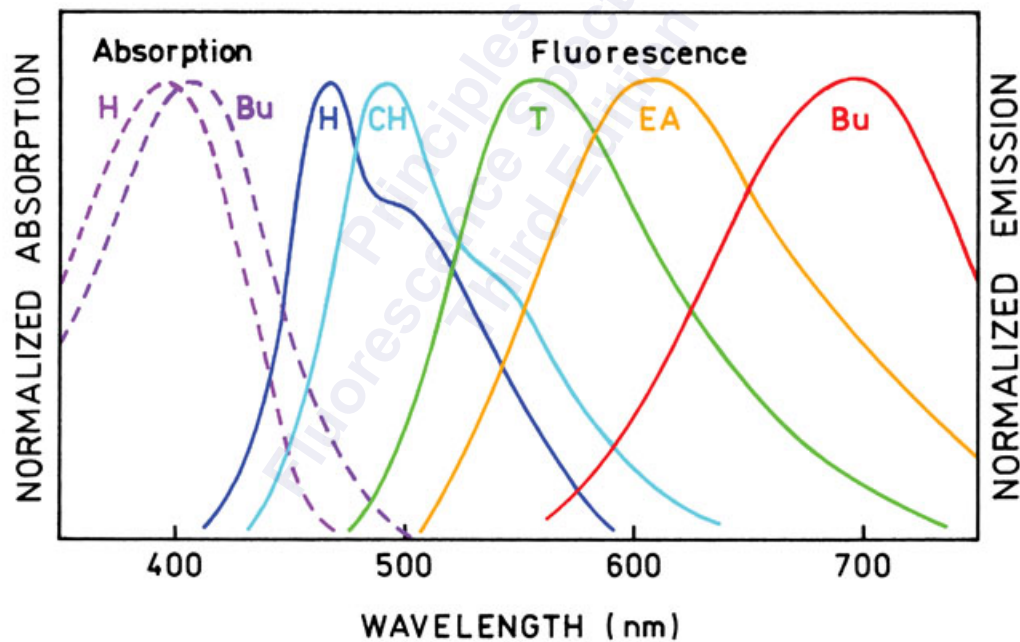












RELATIVE INTENSITY

1.0

0.5

0  
400

500

600

700

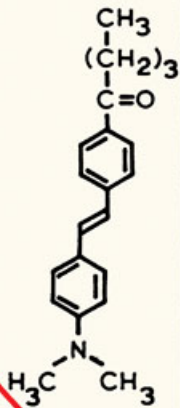
WAVELENGTH (nm)

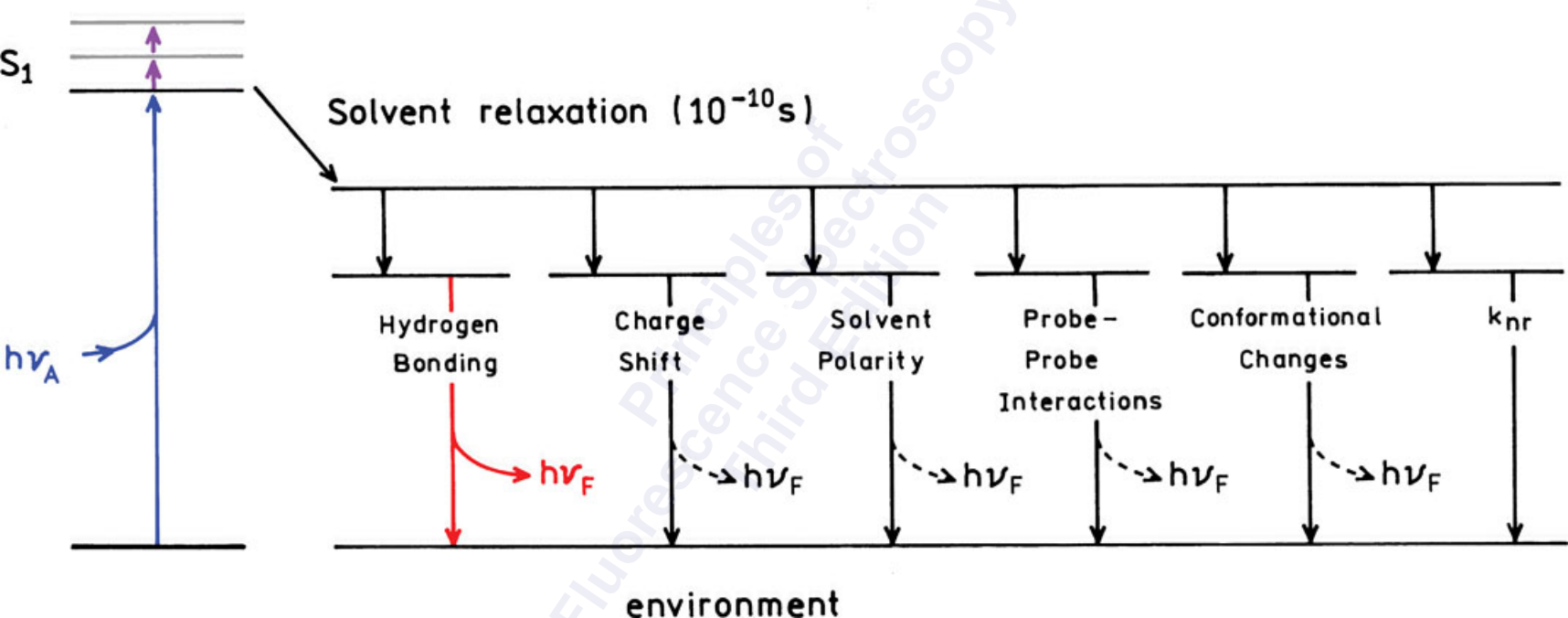
CH

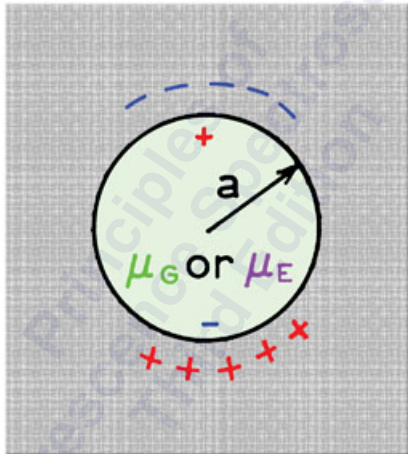
T

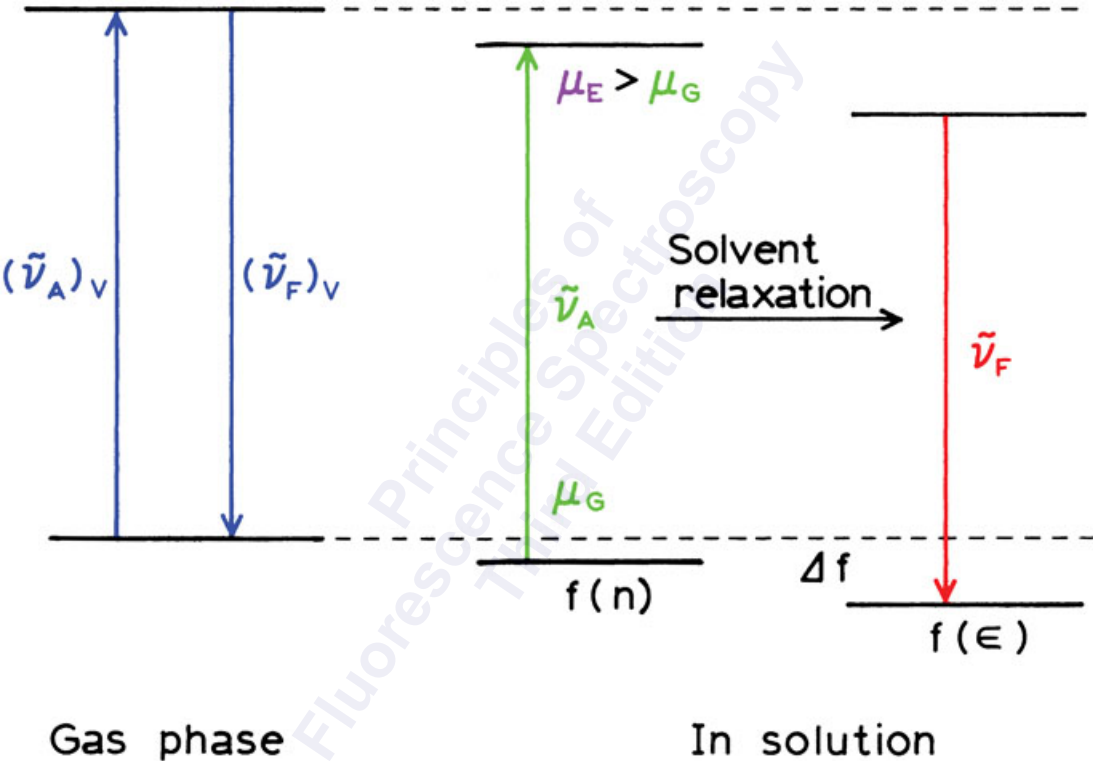
EA

Bu

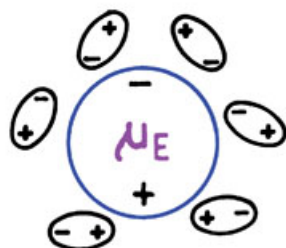






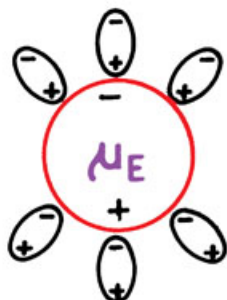


# Franck - Condon State



$R_{or}^G \rightarrow R_{or}^E$   
Solvent Relaxation

## Relaxed State



$h\nu_F$



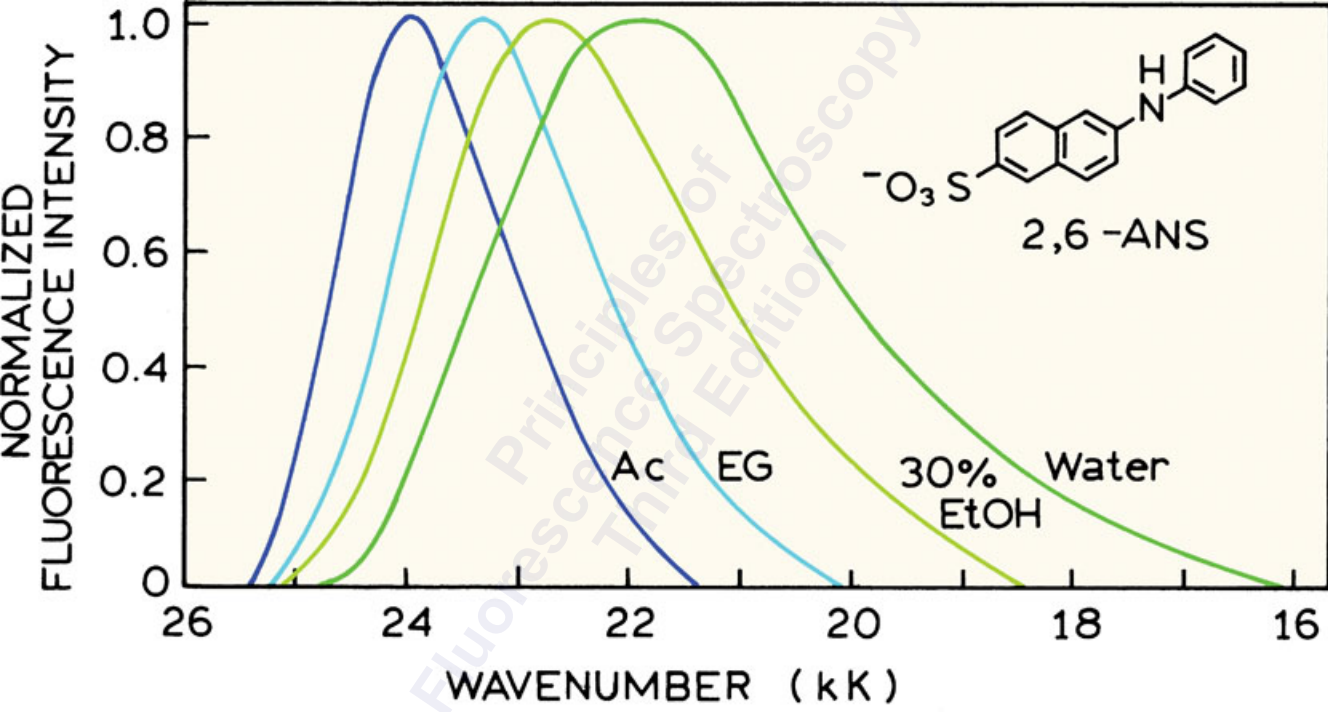
$R_{or}^E \leftarrow R_{or}^G$

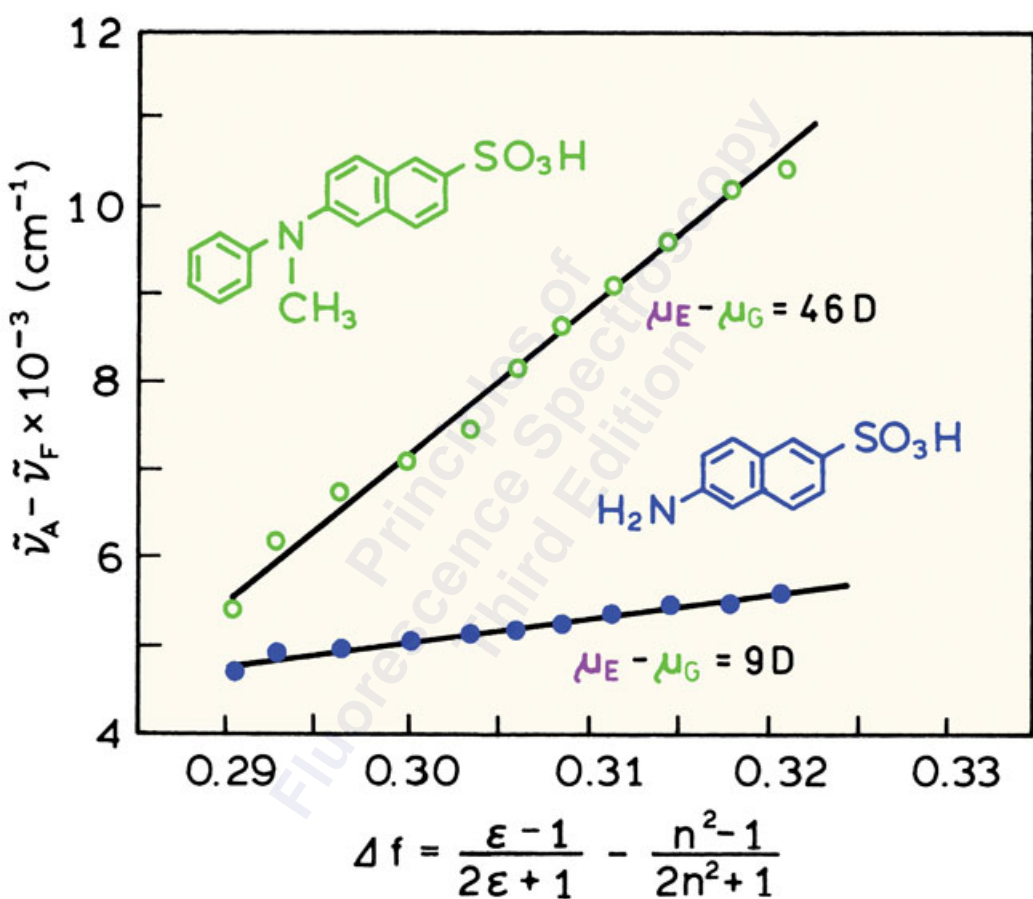


Franck - Condon Principle  
 $R_{or}^G$  is unchanged

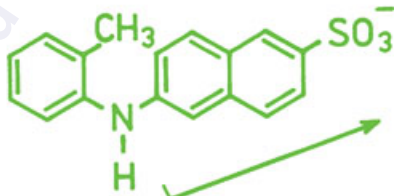
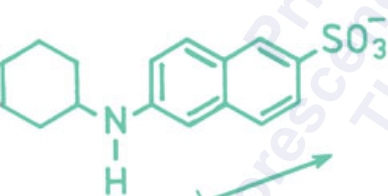
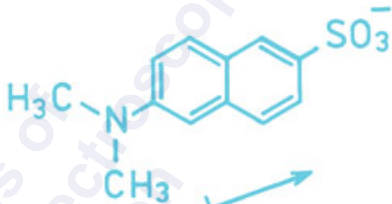
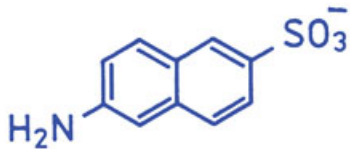
$h\nu_A$

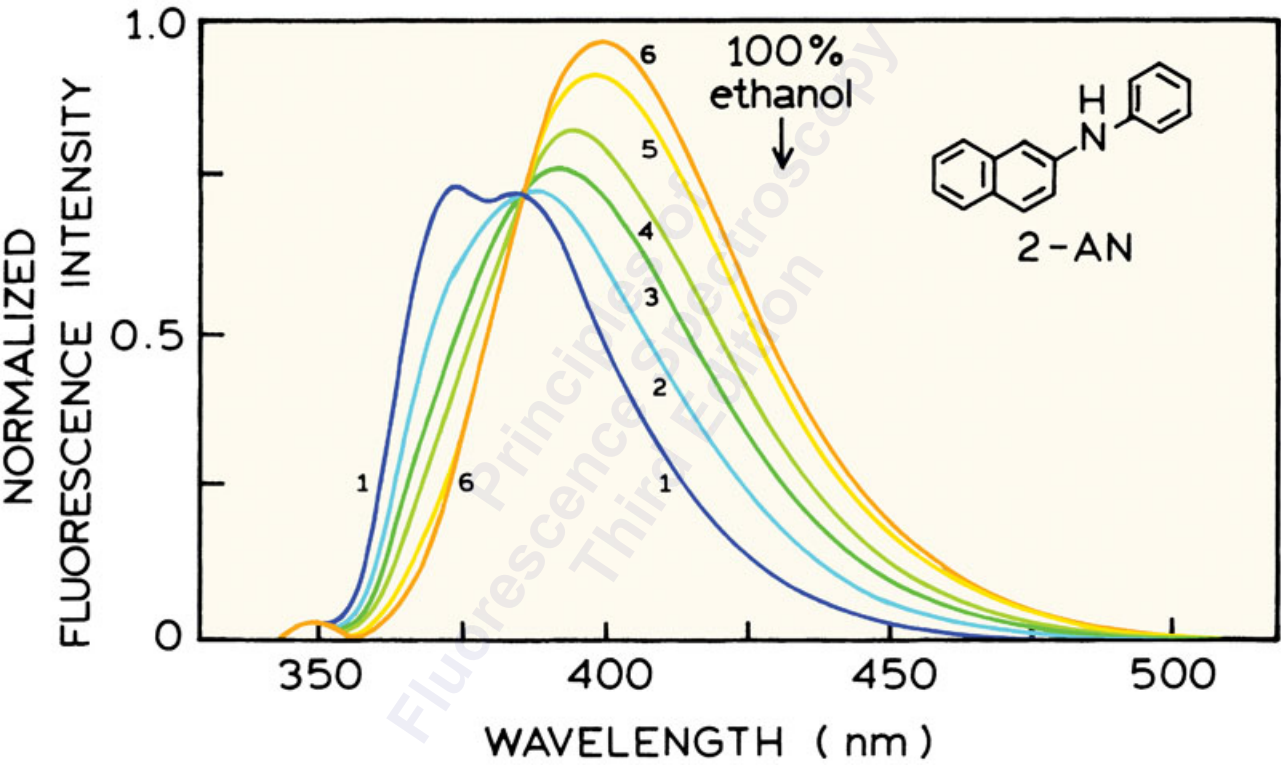


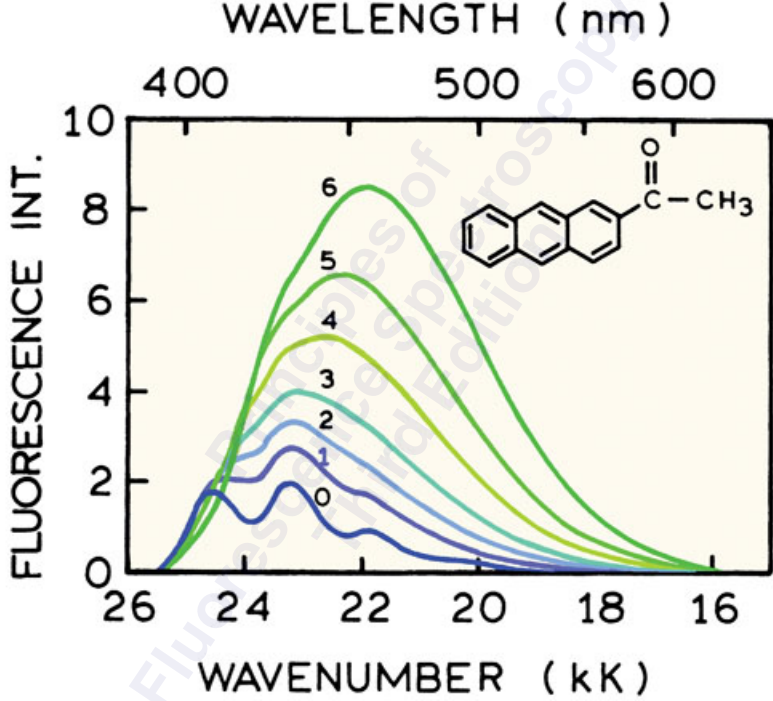




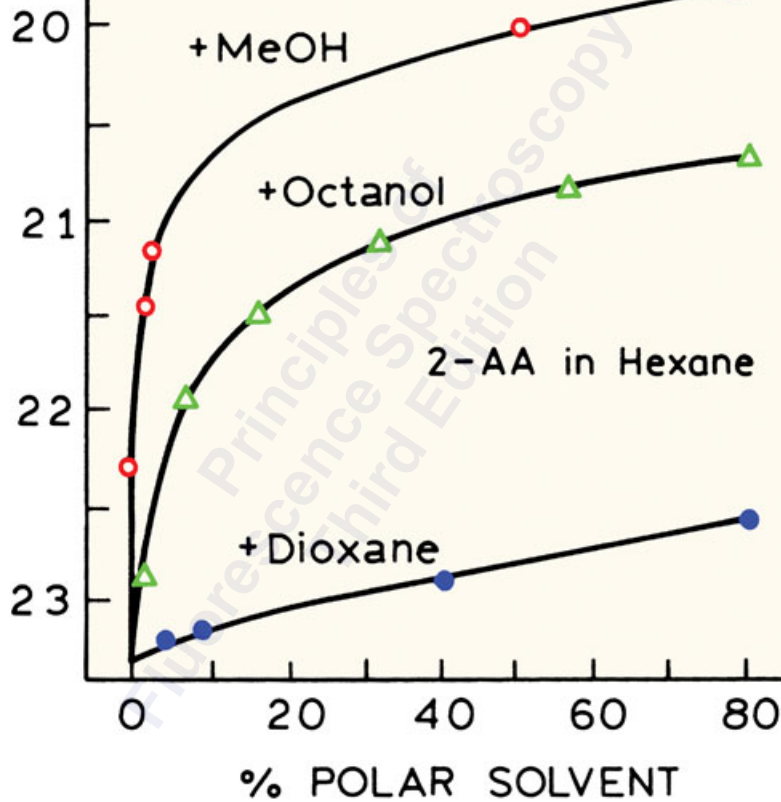


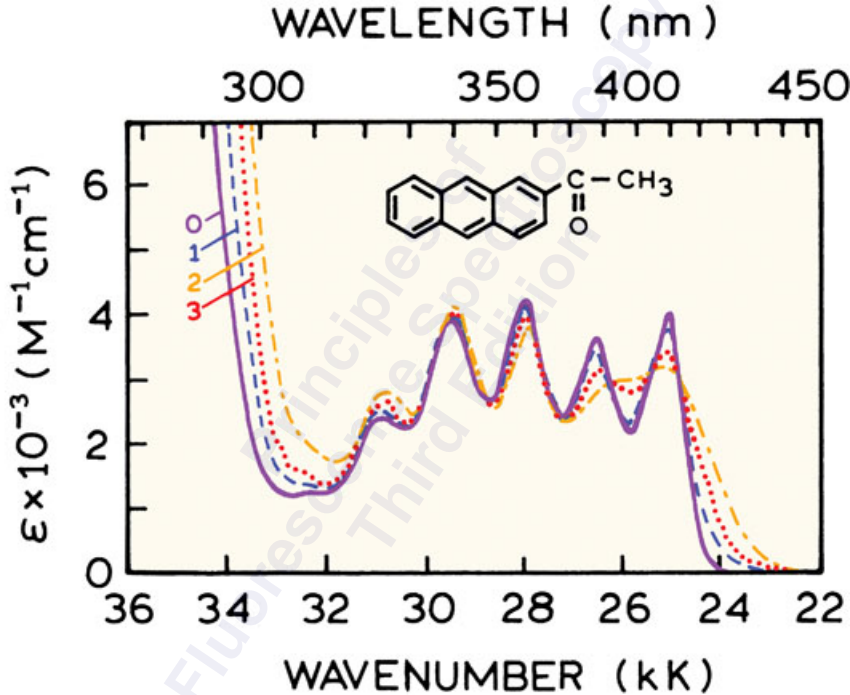


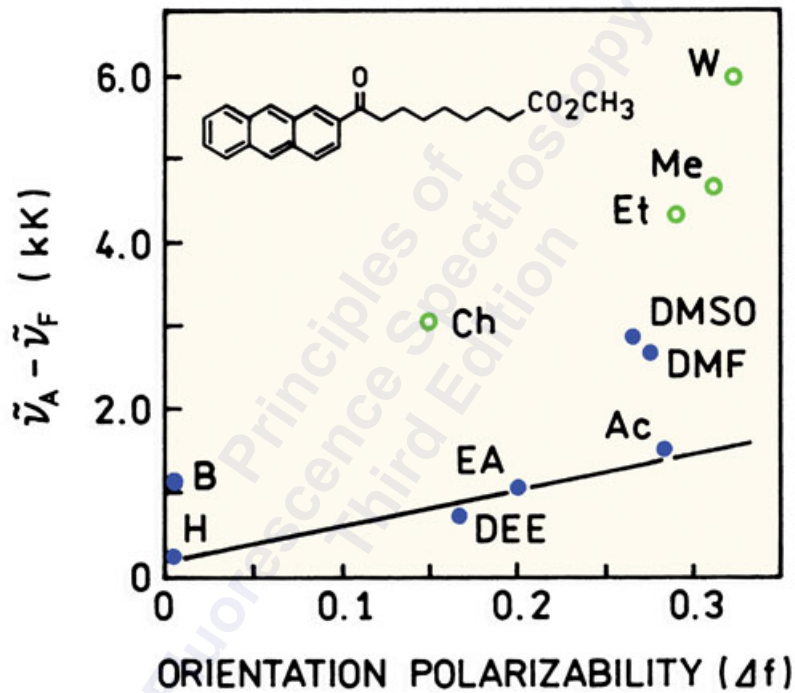




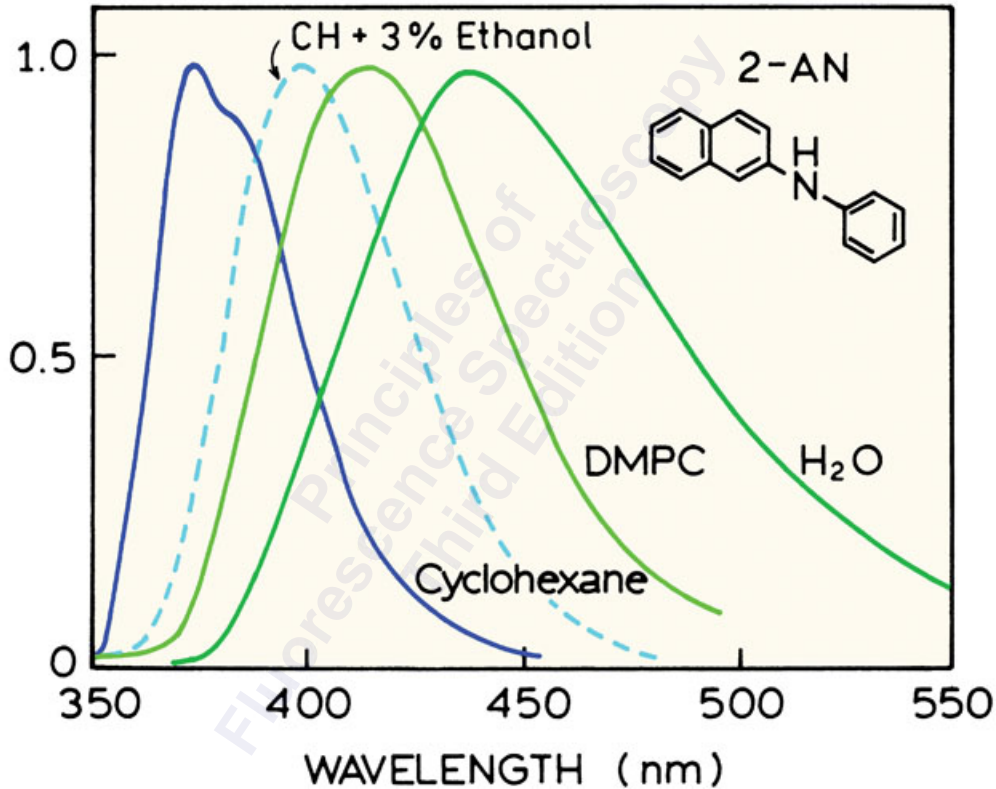
EMISSION MAXIMUM ( kK )

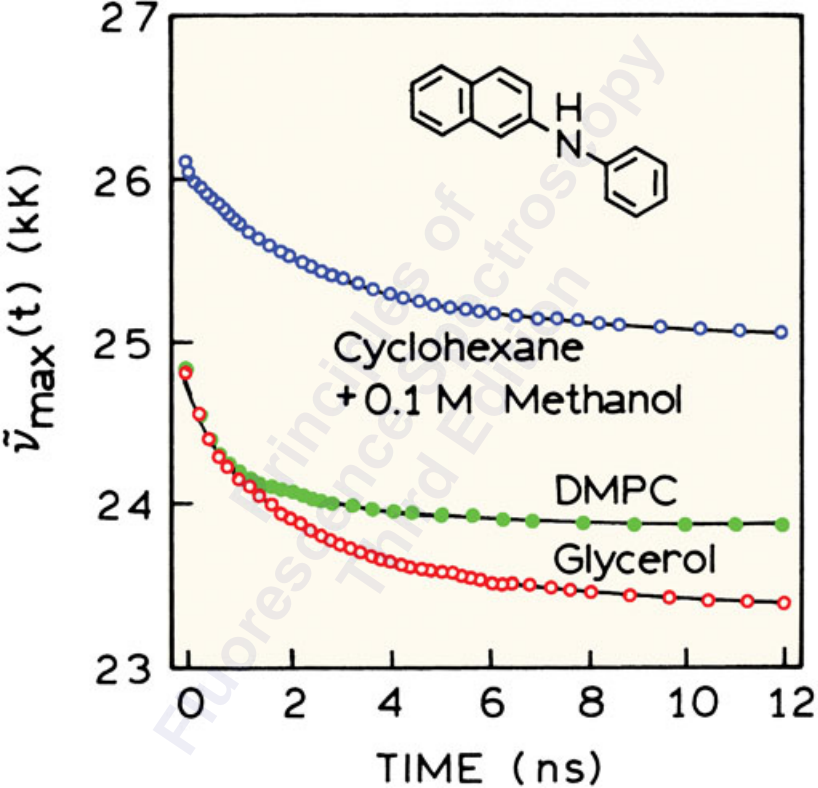




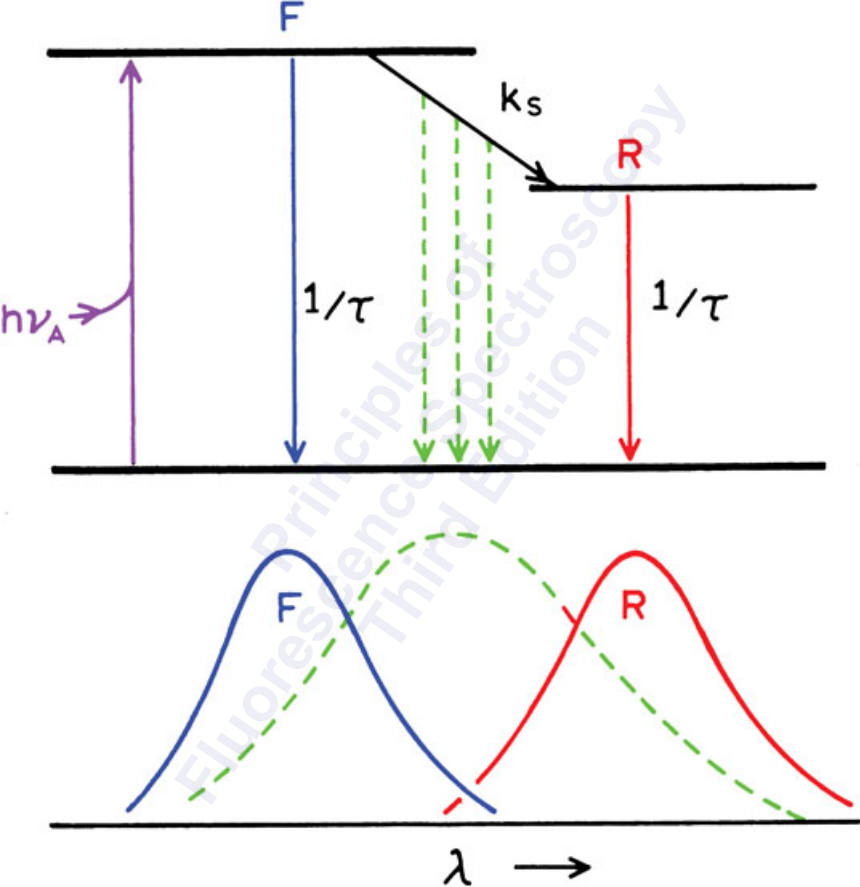


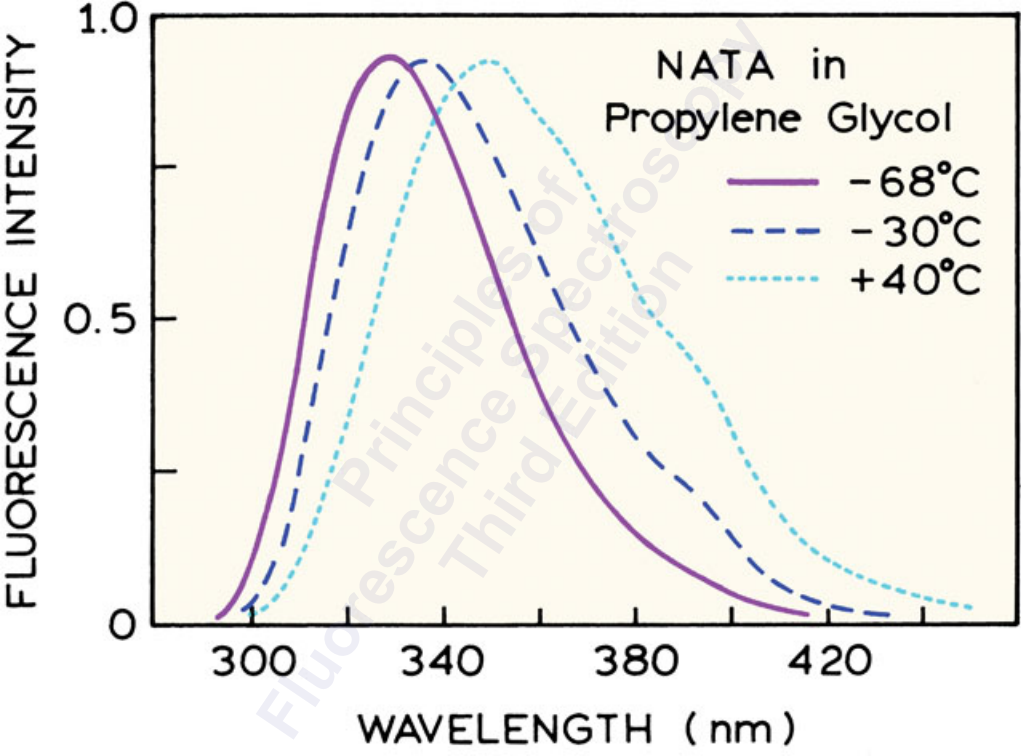
FLUORESCENCE INTENSITY

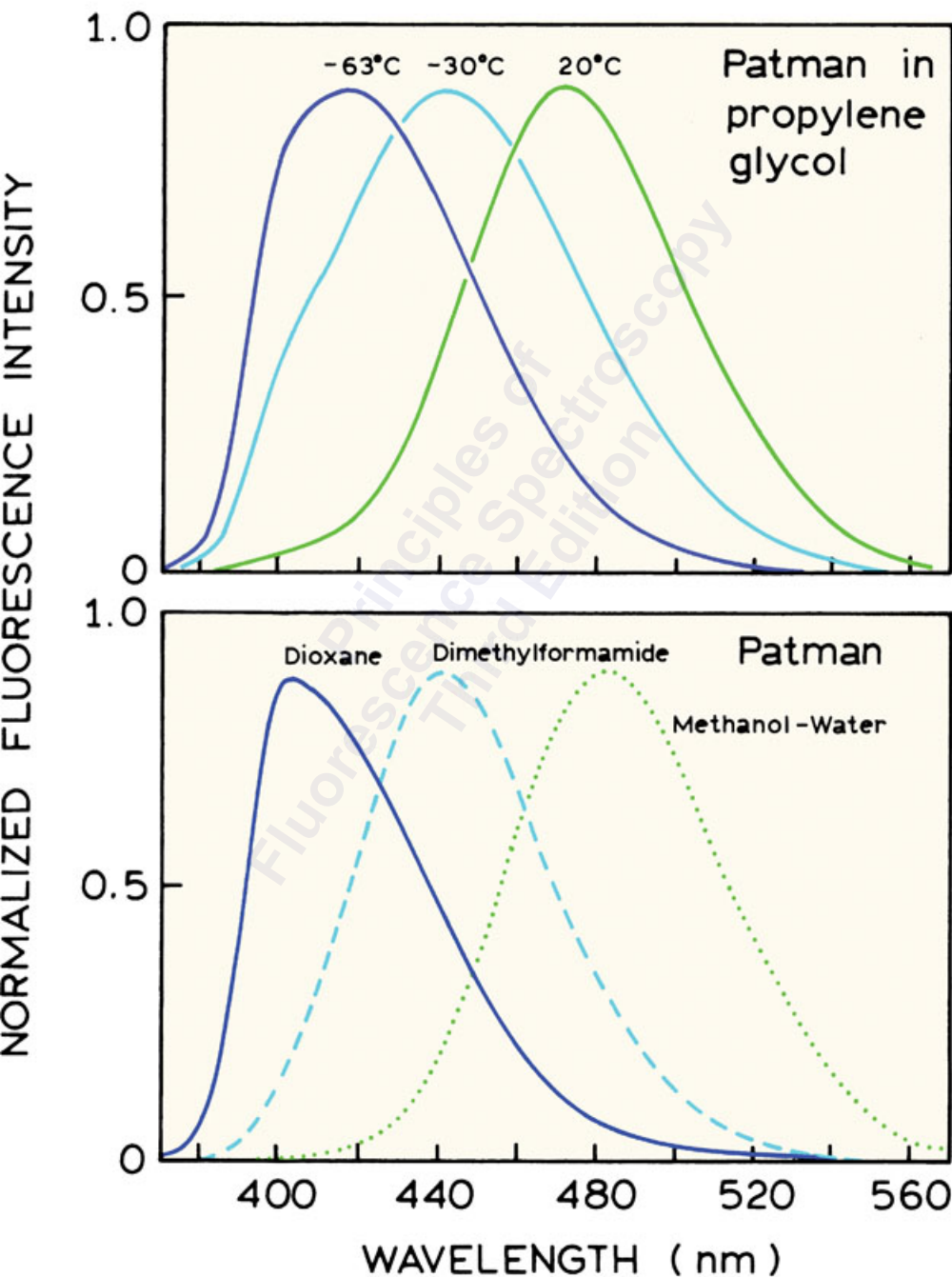
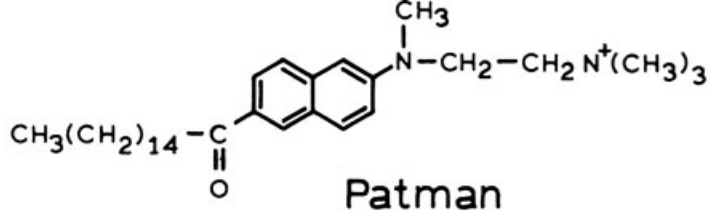


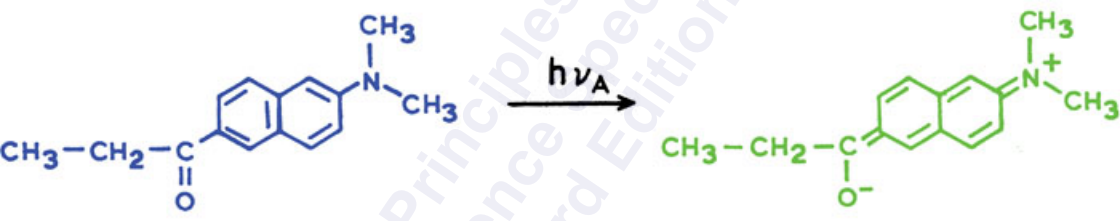


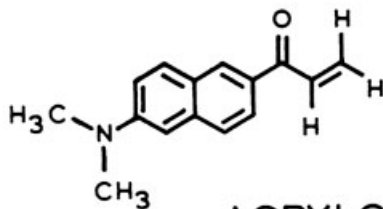
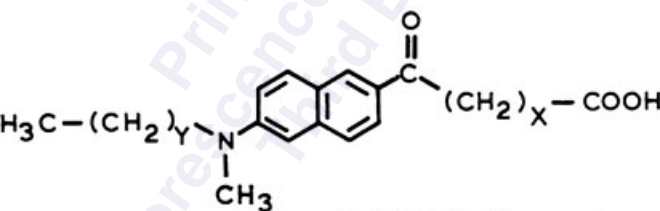
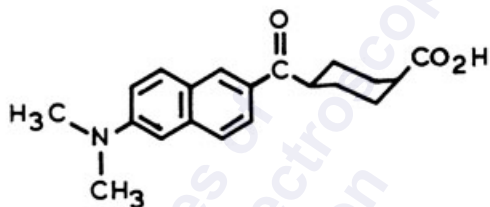
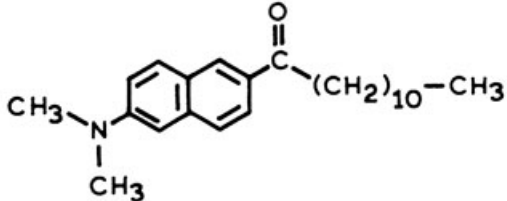


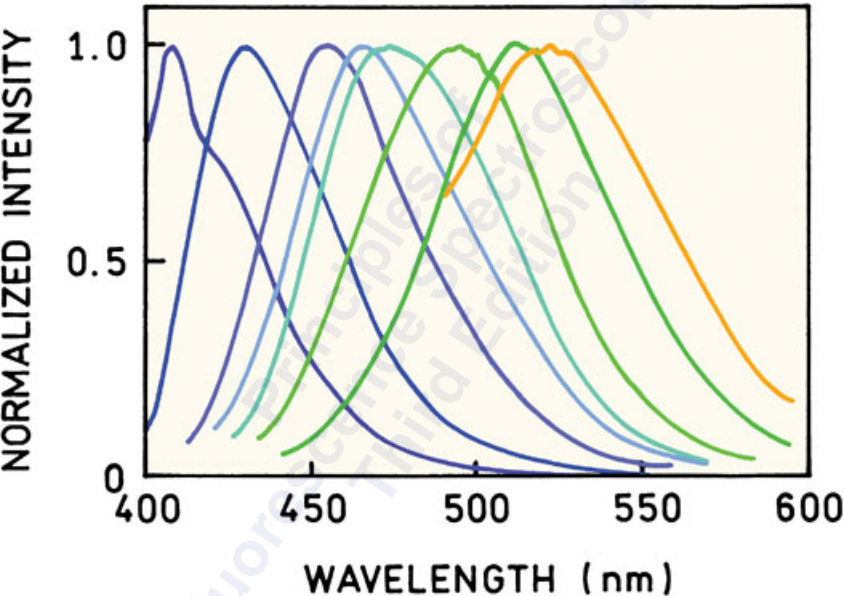




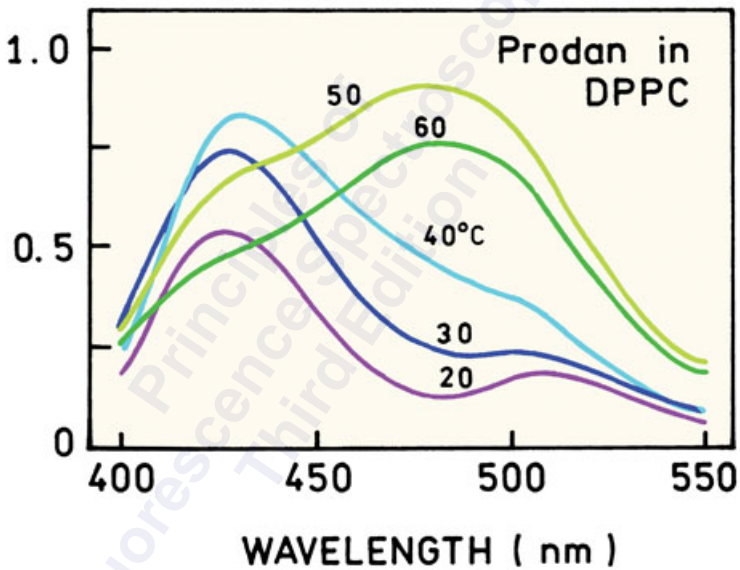








FLUORESCENCE  
INTENSITY



63°C



54°C



40°C



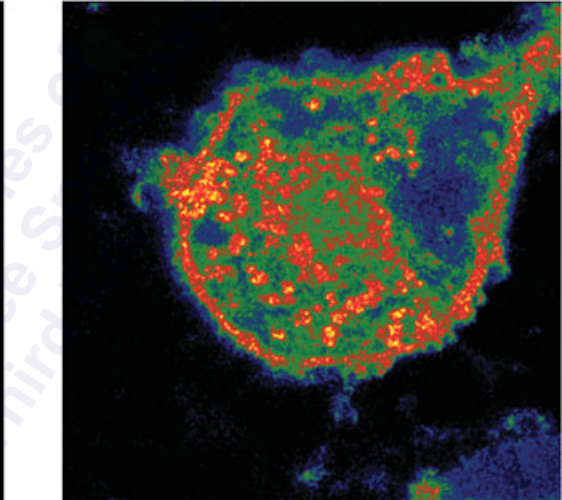
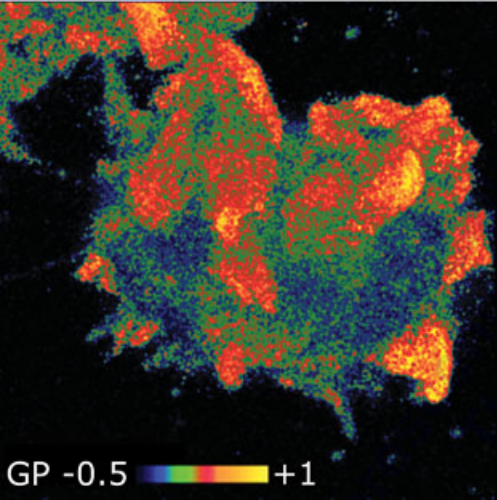
-0.8

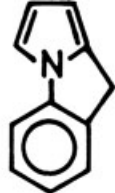
GP

0.8

30  $\mu\text{m}$





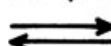


FPP



LE

12 ps



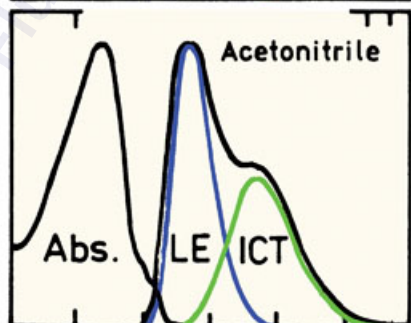
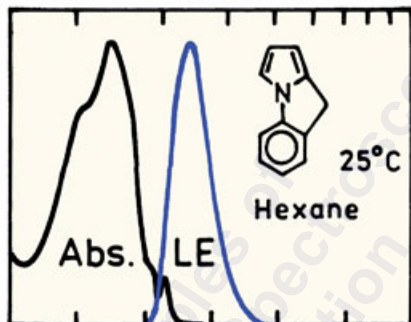
ICT

$\mu_e(\text{LE}) = 1\text{D}$

$\mu_e(\text{ICT}) = 13\text{D}$

WAVELENGTH (nm)

250 350 450 650



ABSORBANCE

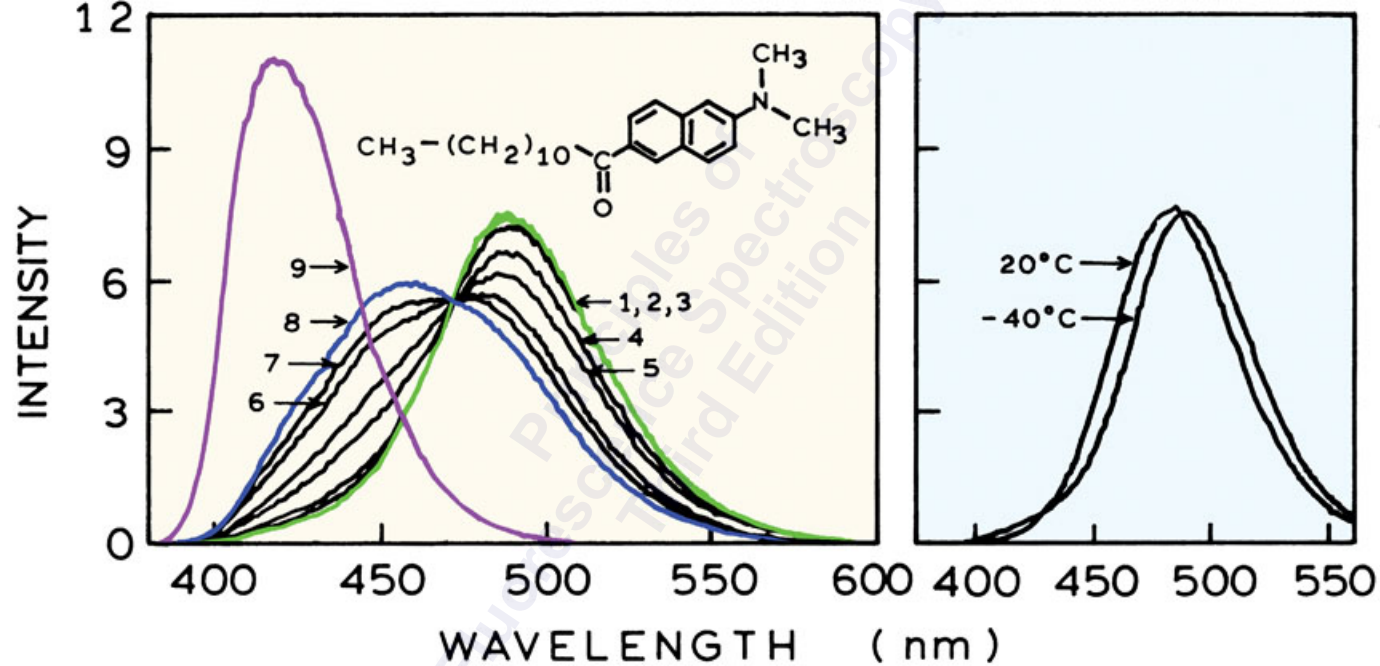
FLUORESCENCE INTENSITY

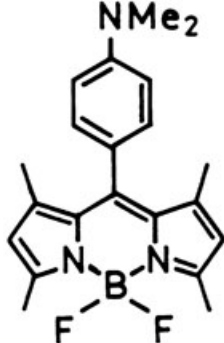
WAVENUMBER (kK)

40

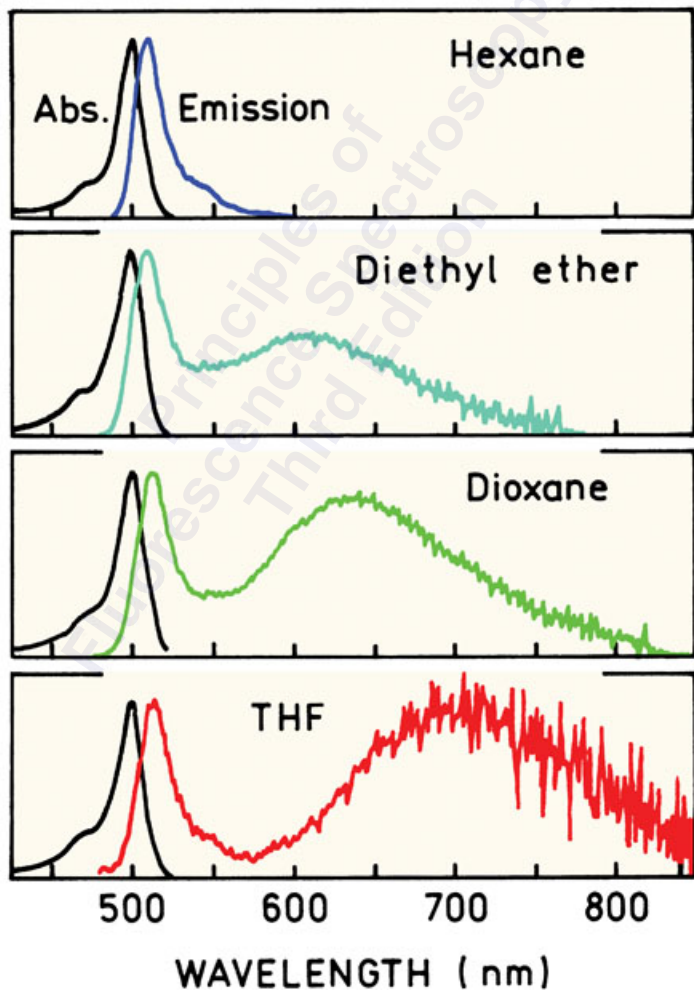
30

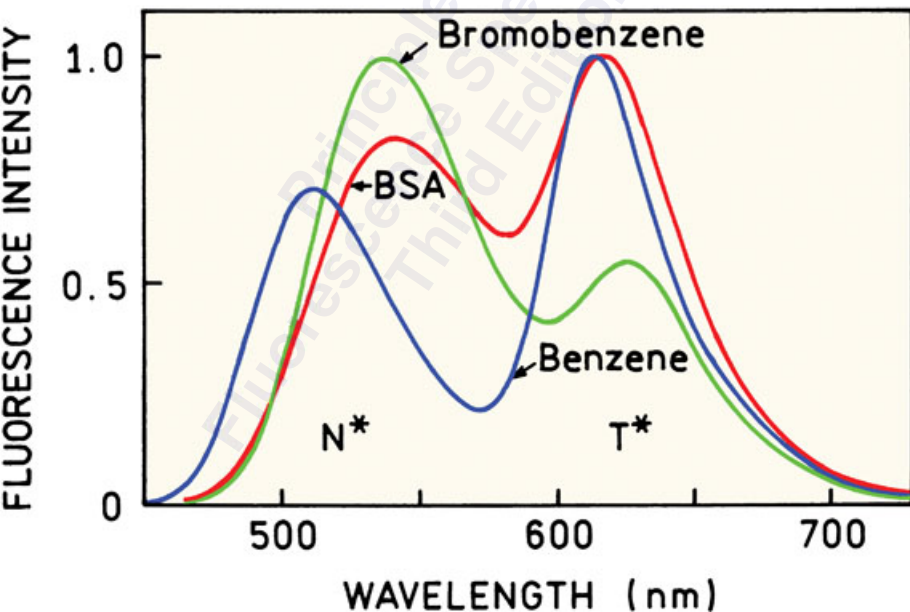
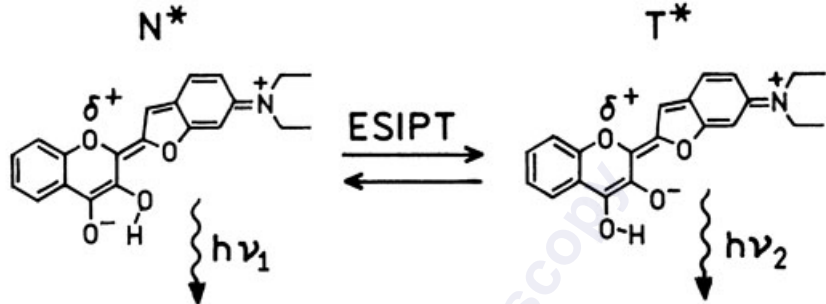
20

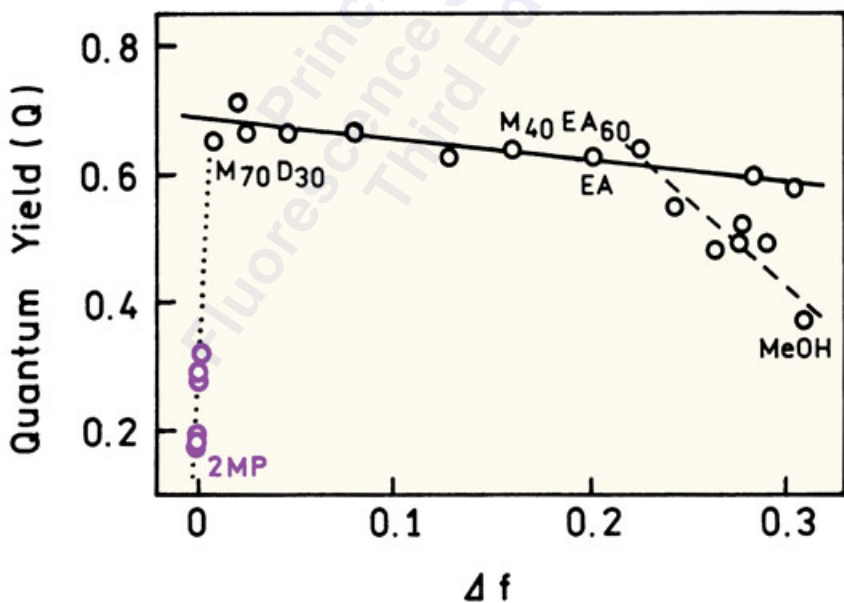
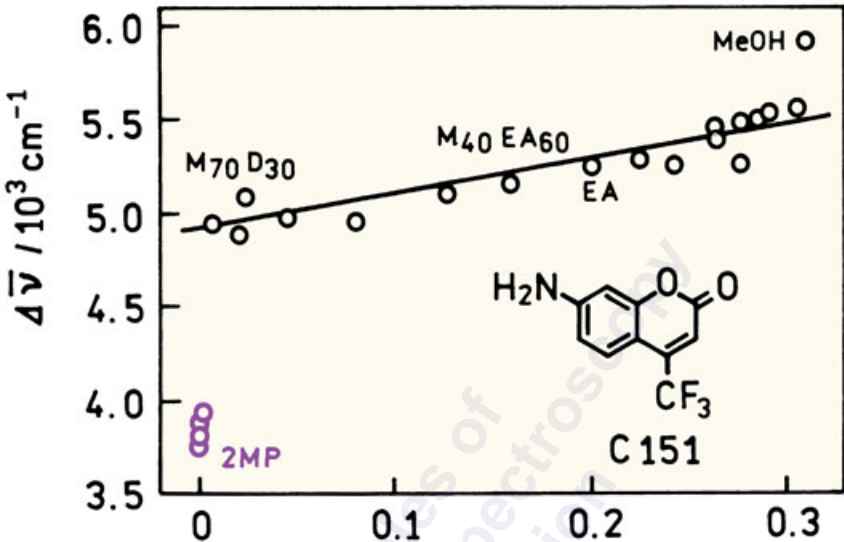


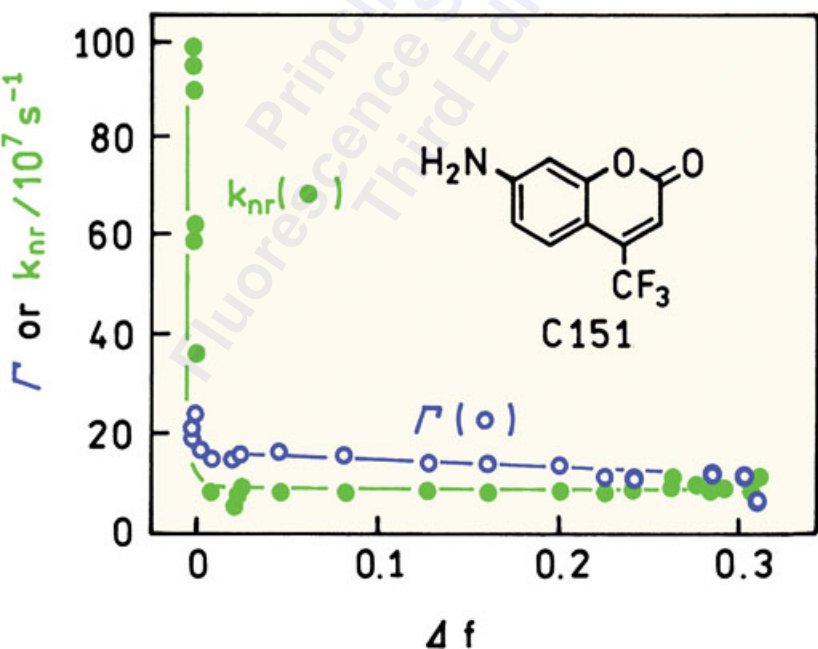
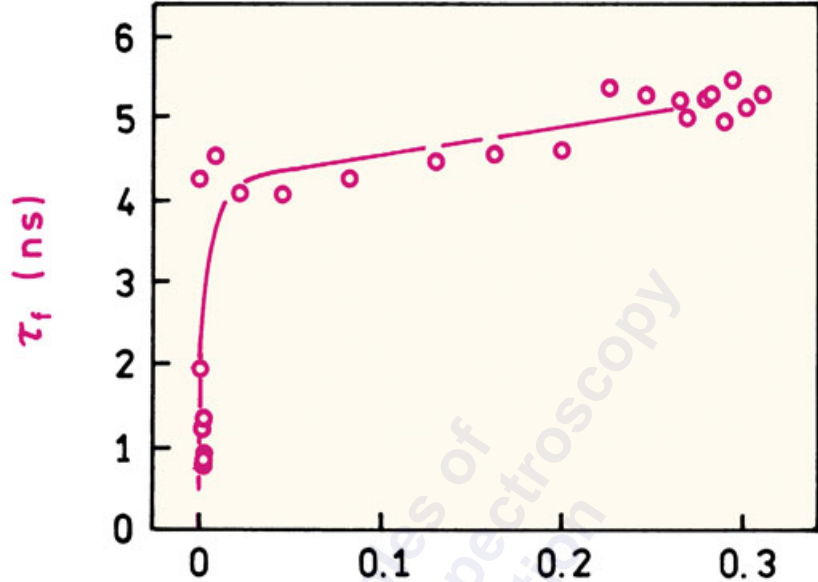


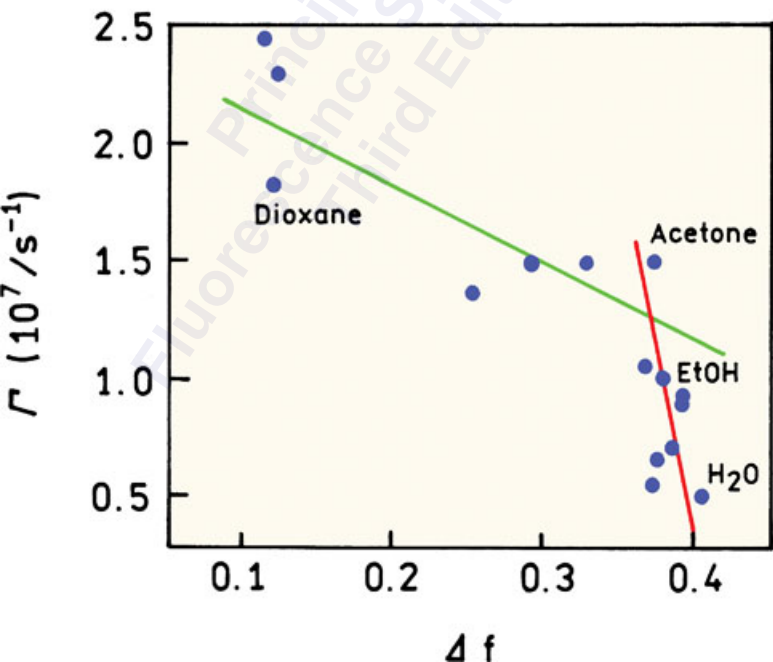
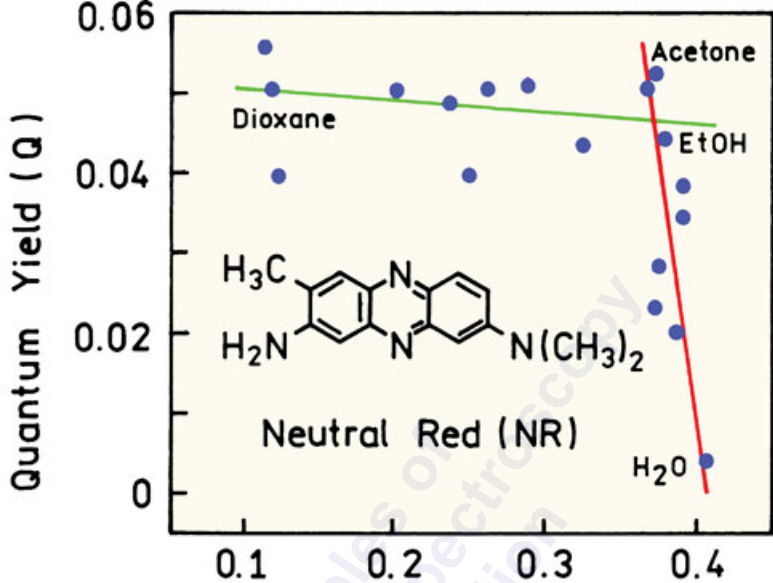
ABSORPTION or EMISSION INTENSITY





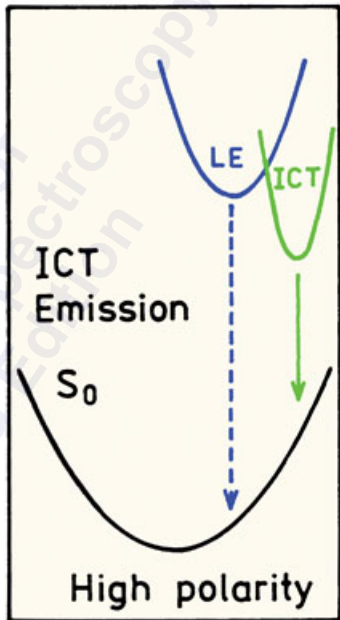
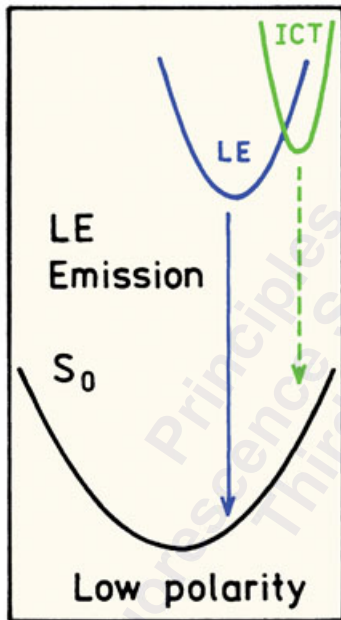




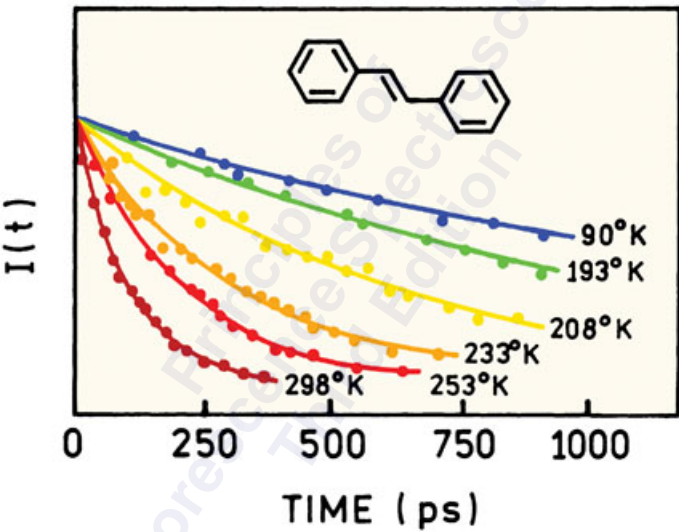


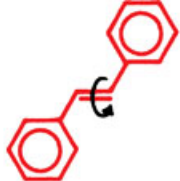


Energy



Solvation Coordinate

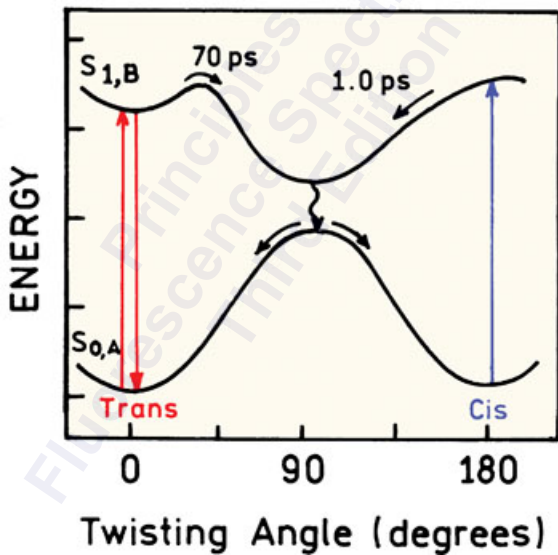




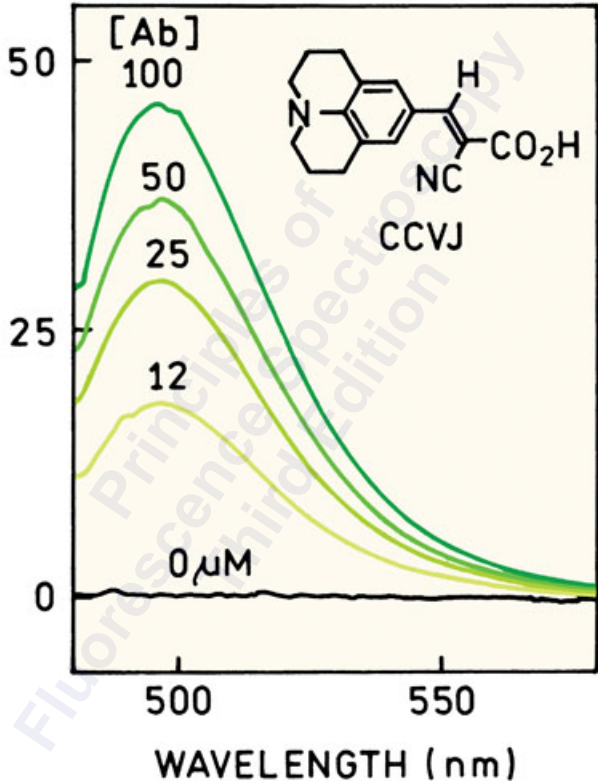
Trans - Stilbene

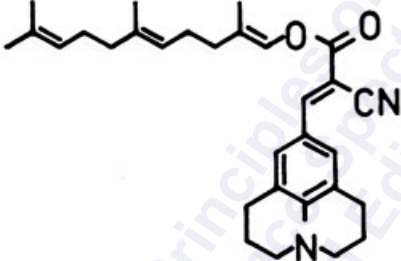


Cis - Stilbene

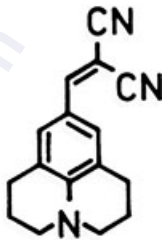


FLUORESCENCE INTENSITY

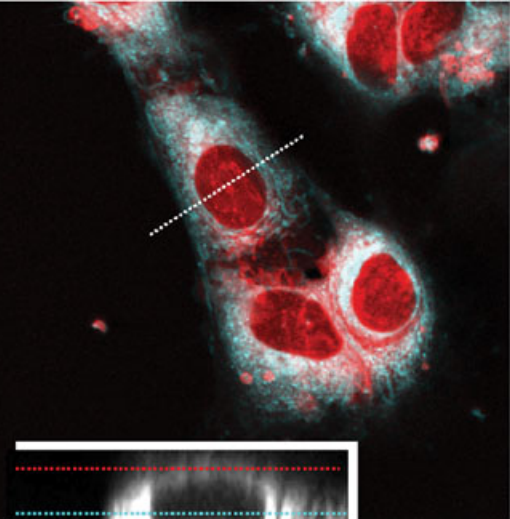




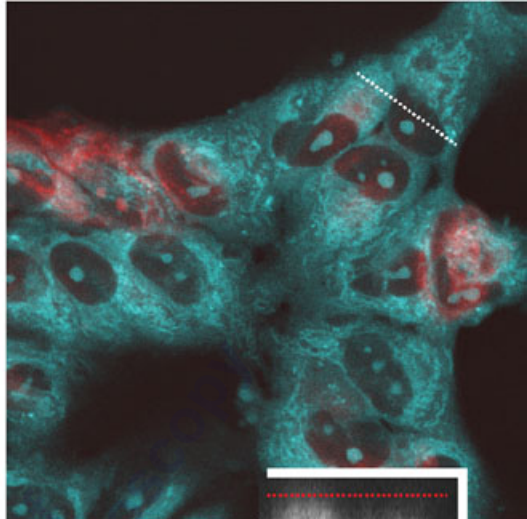
FCVJ



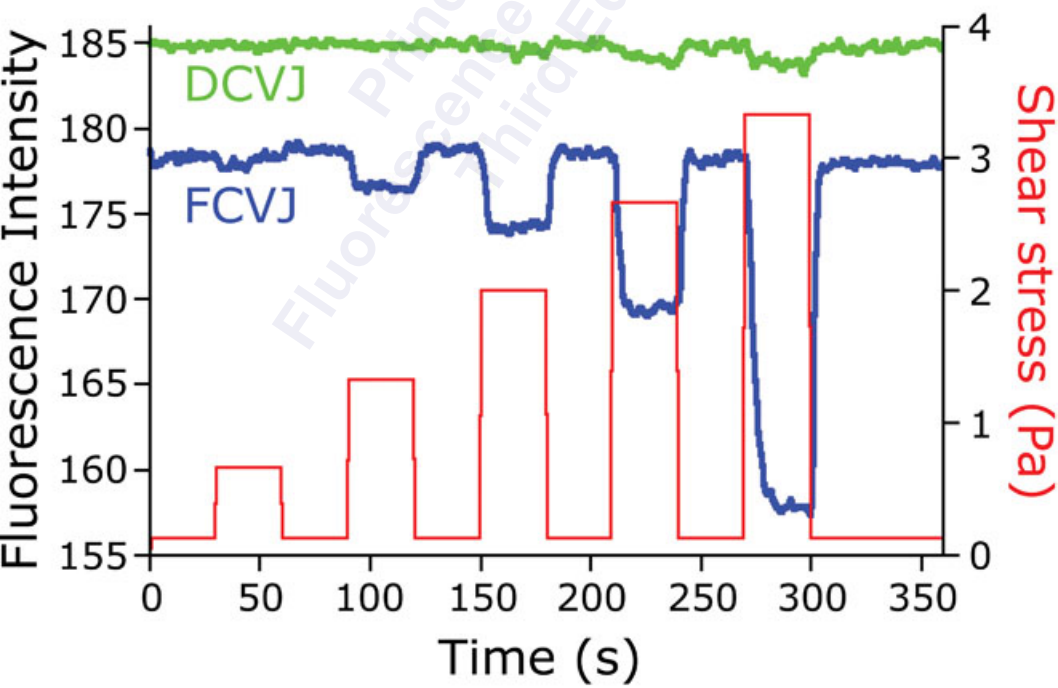
DCVJ

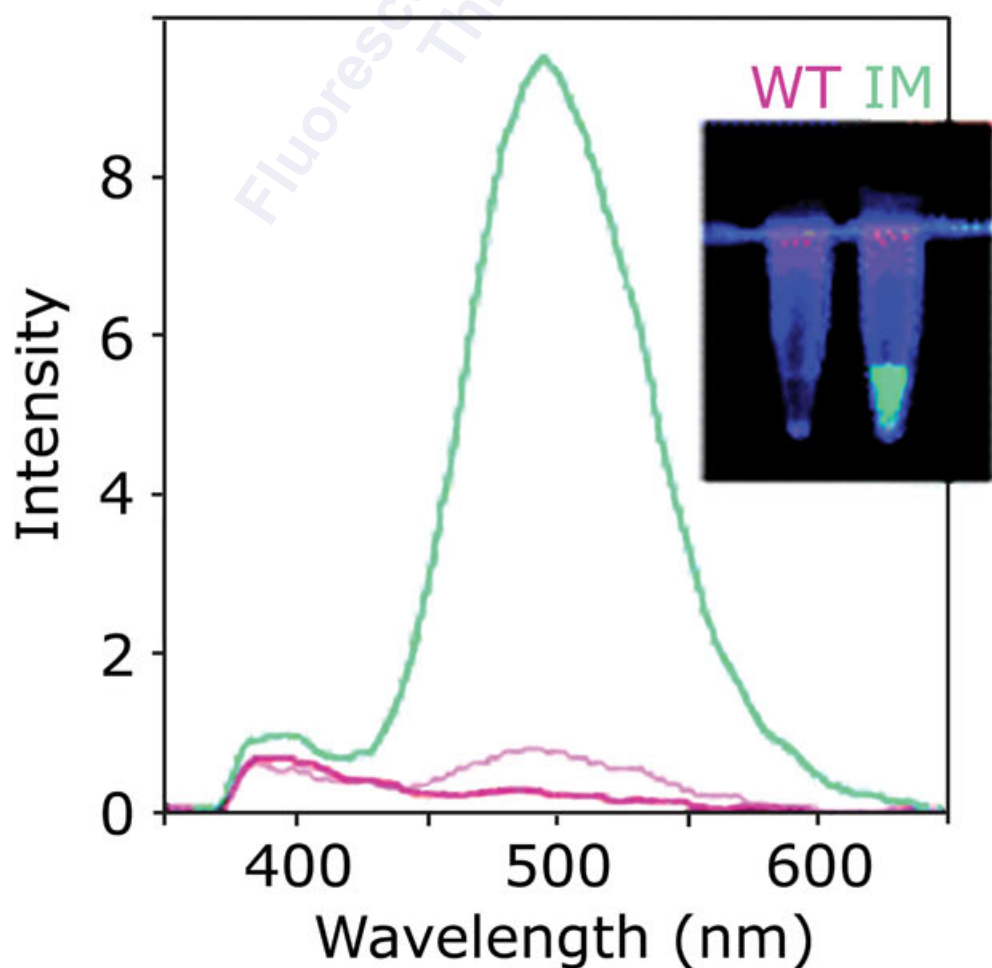
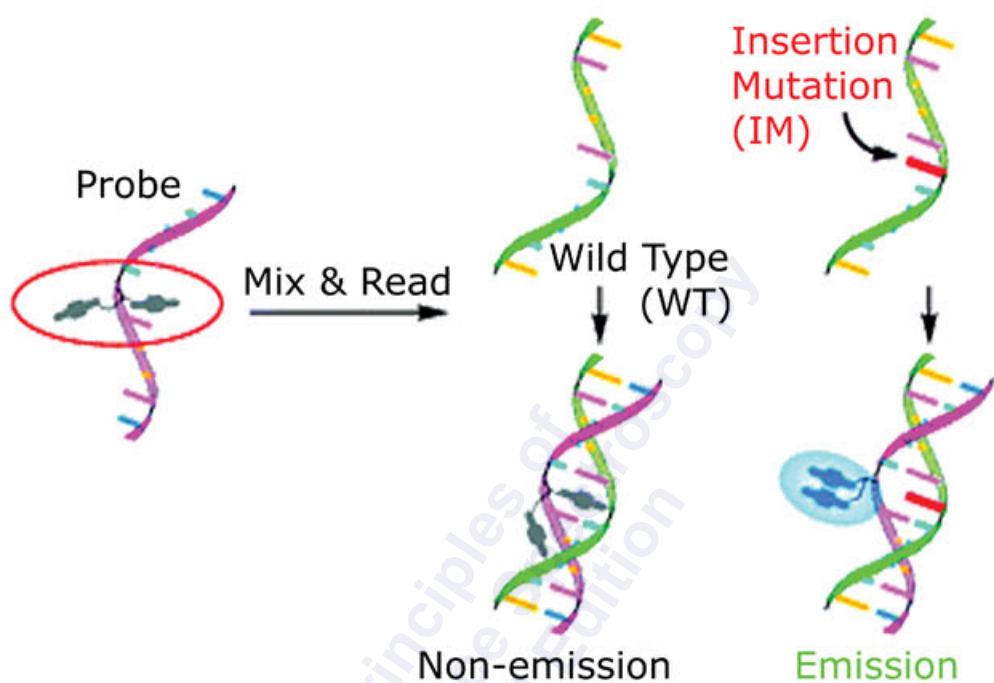
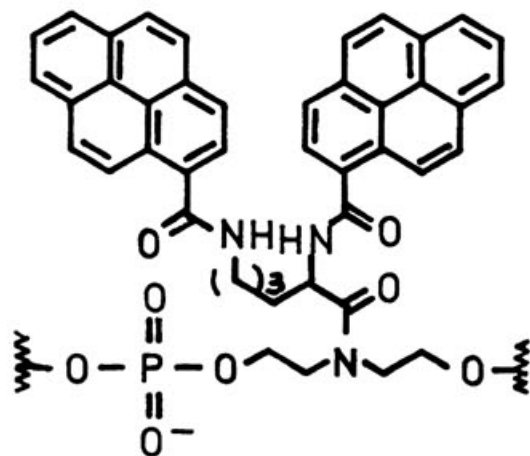


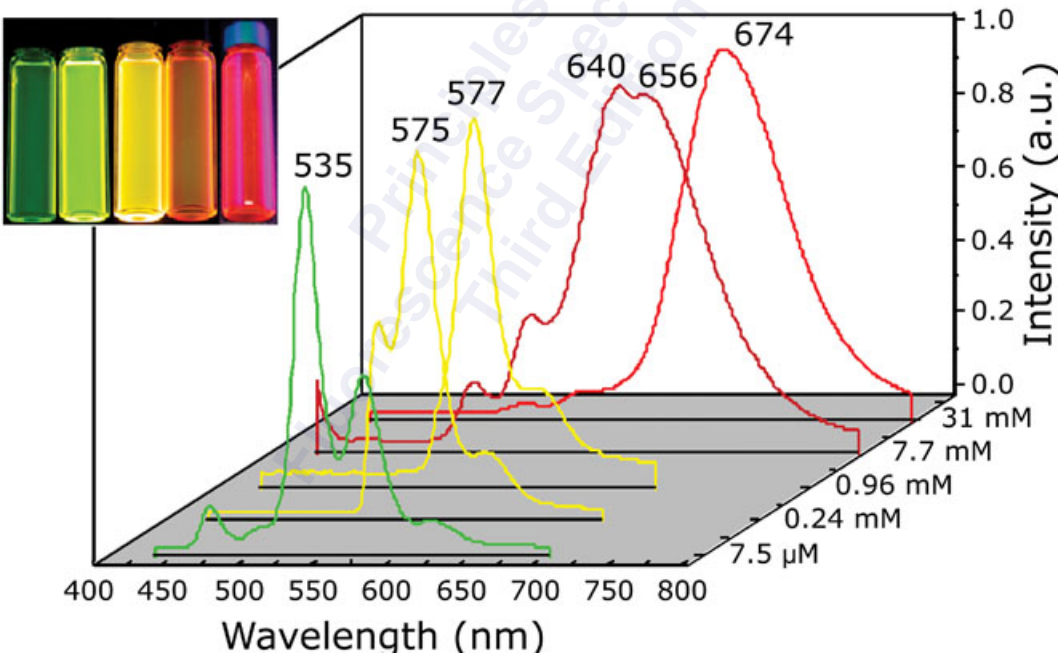
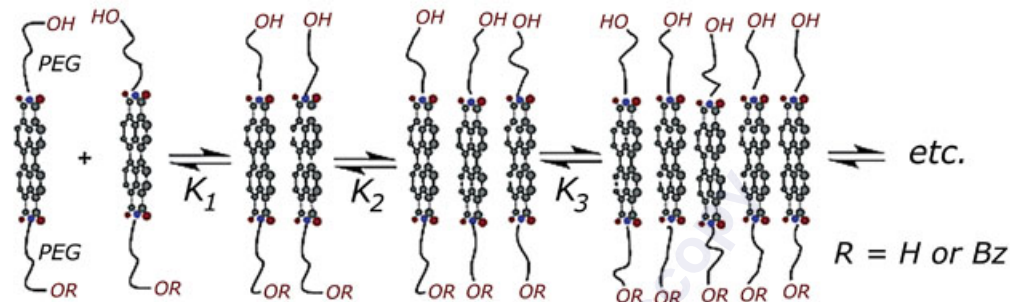
FCVJ



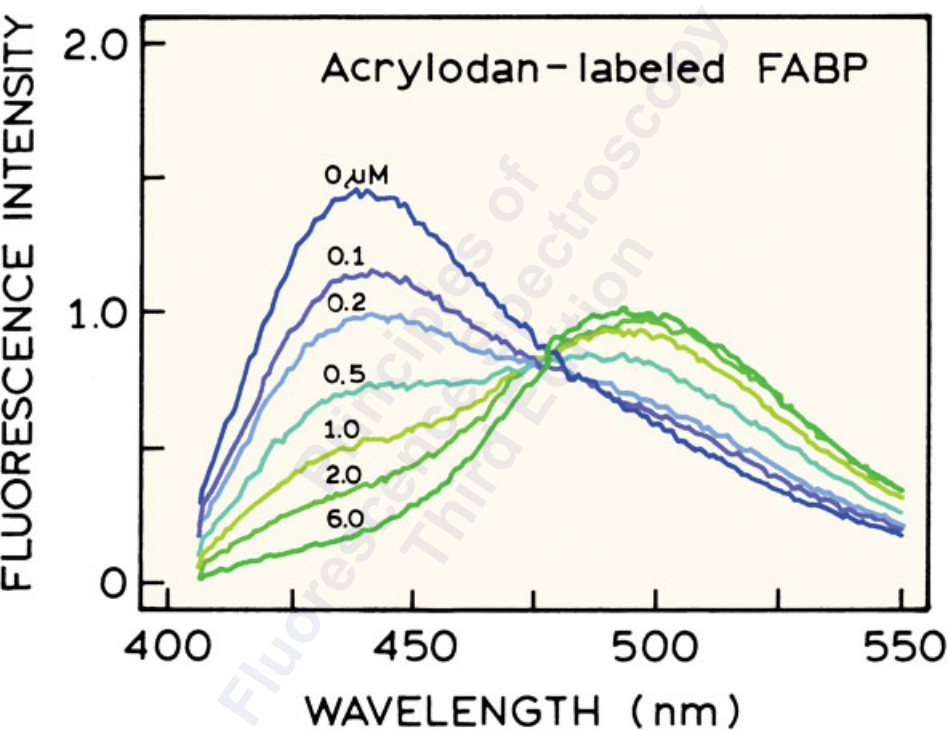
DCVJ



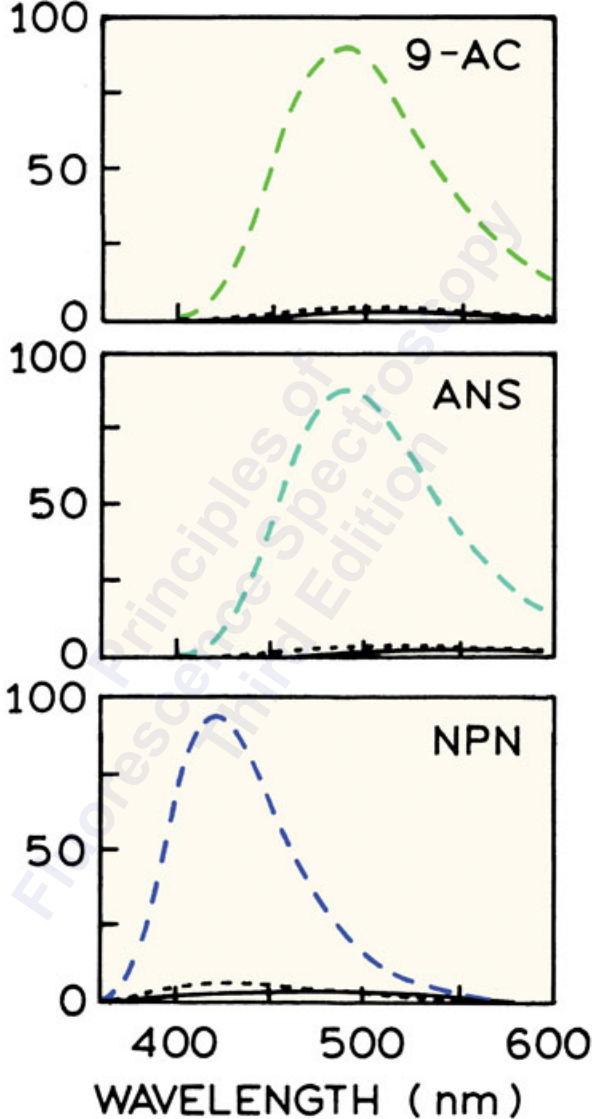


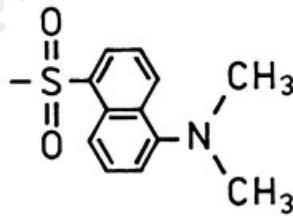
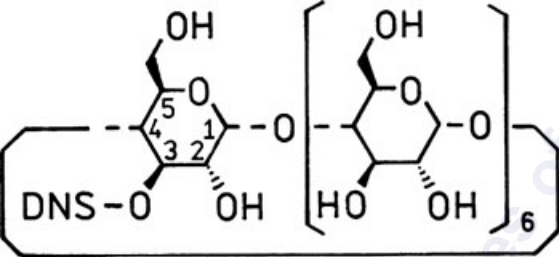




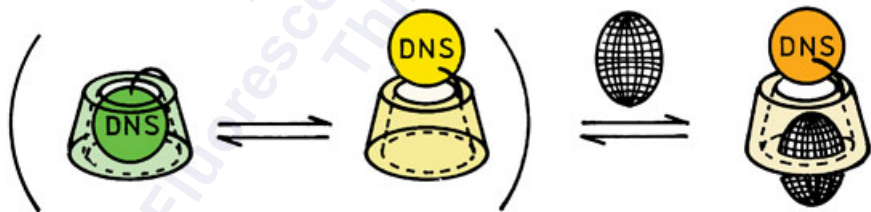


RELATIVE FLUORESCENCE INTENSITY

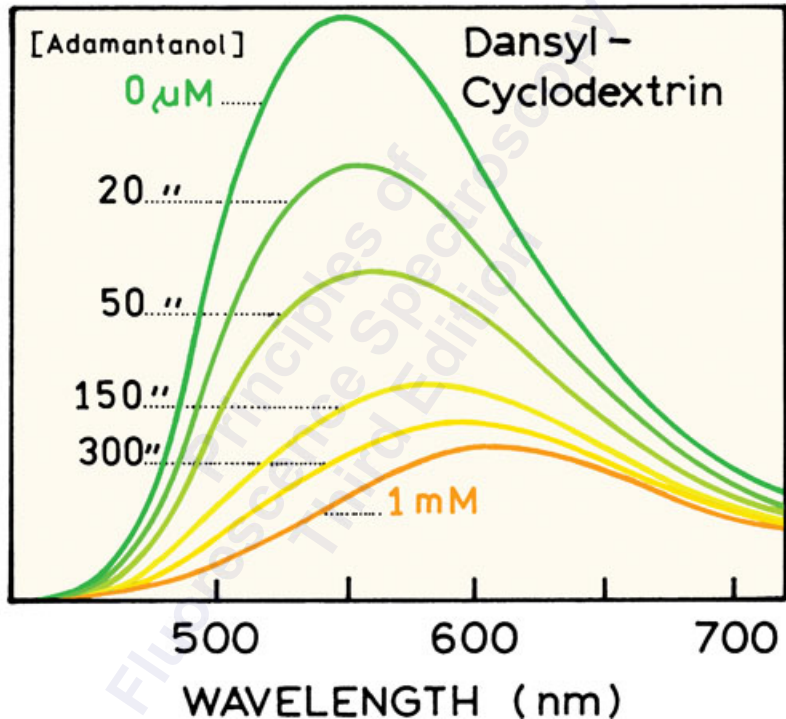




DNS

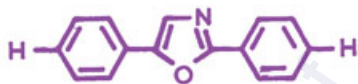


REL. FLUORESCENCE INTENSITY



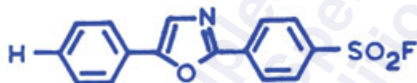
DONOR

ACCEPTOR

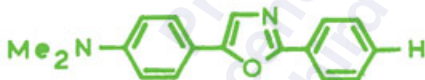


A

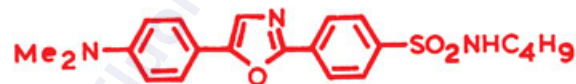
DPO



B

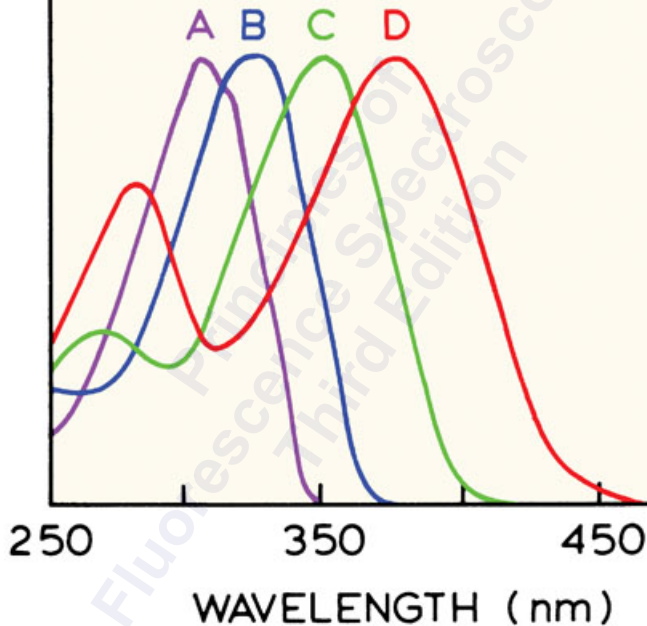


C

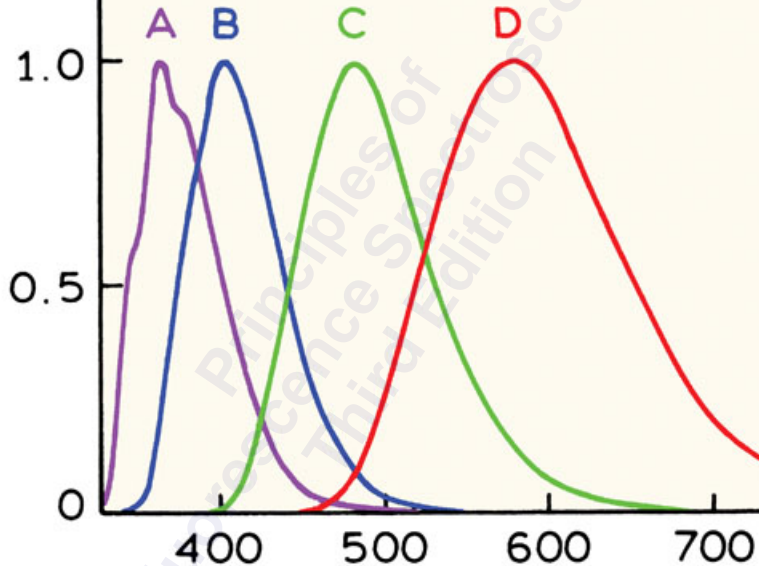


D

ABSORBANCE

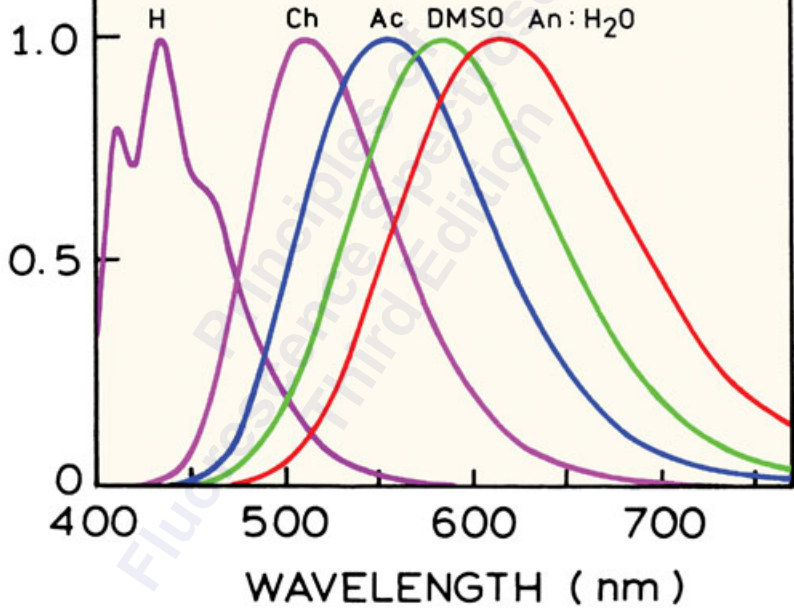
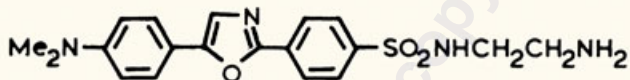


FLUORESCENCE INTENSITY

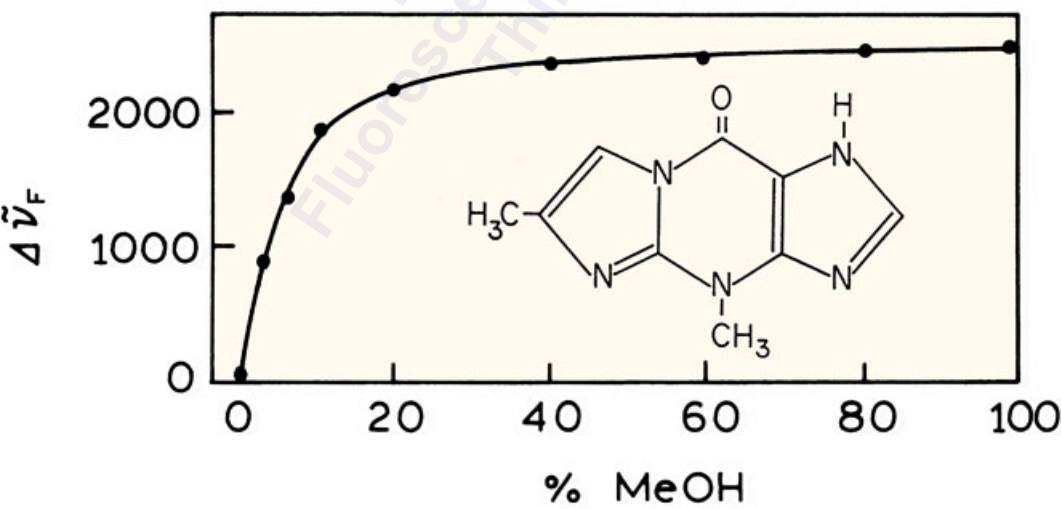
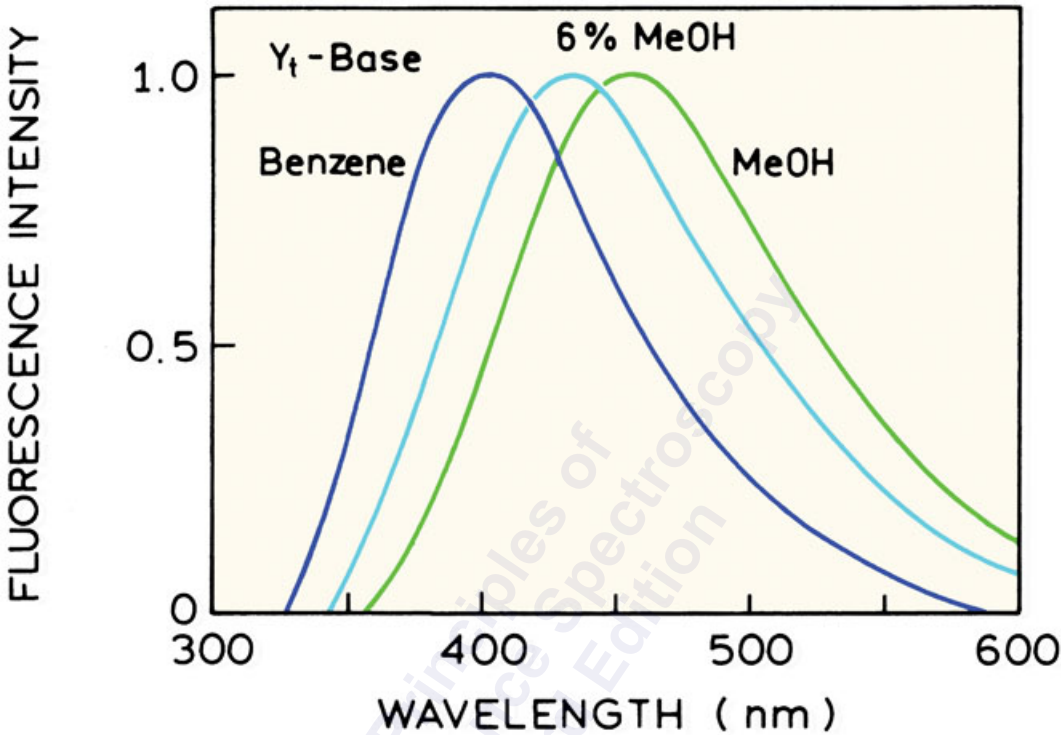


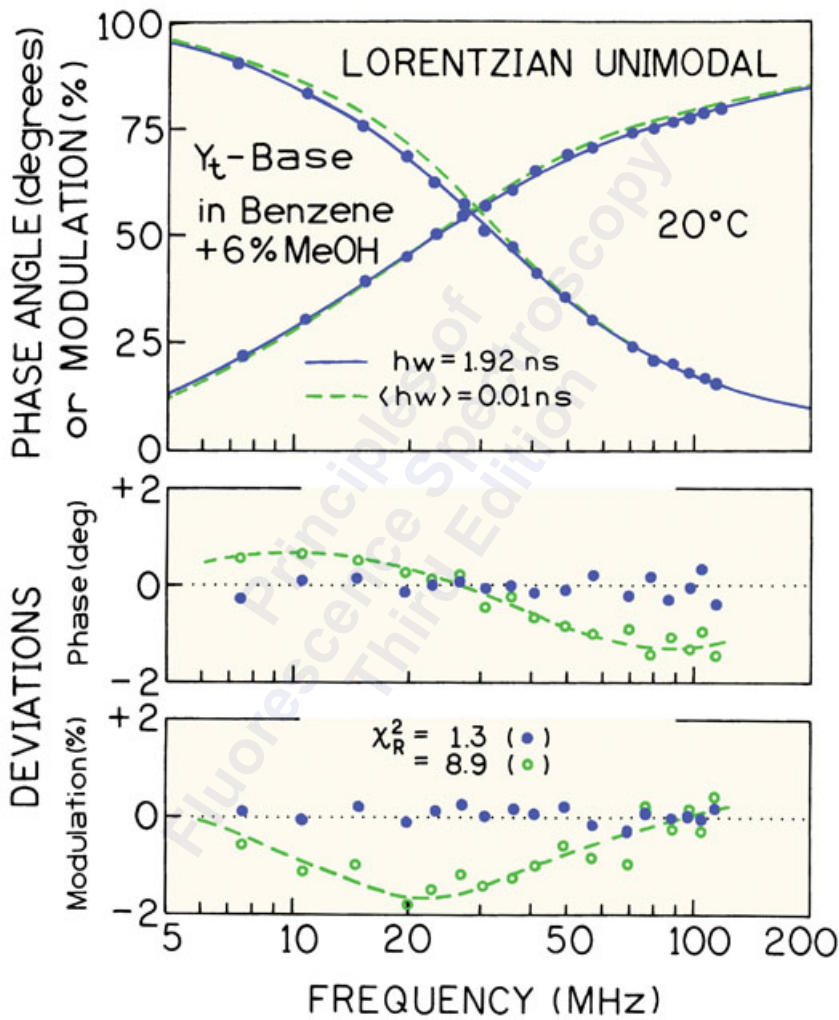
WAVELENGTH ( nm )

FLUORESCENCE INTENSITY

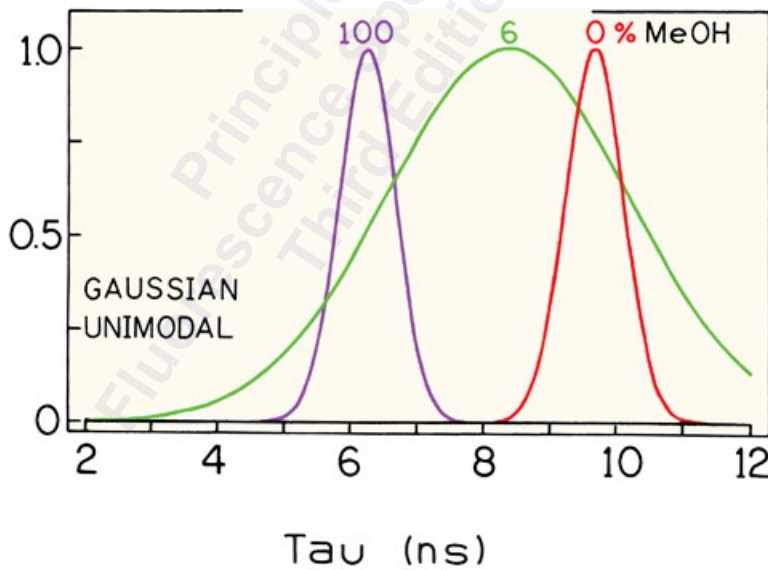
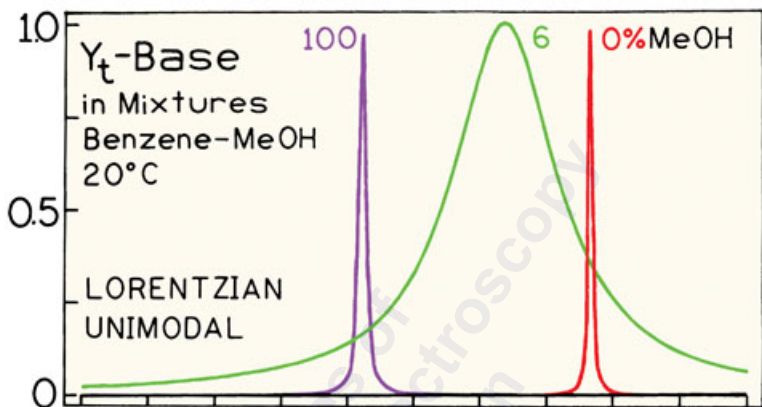


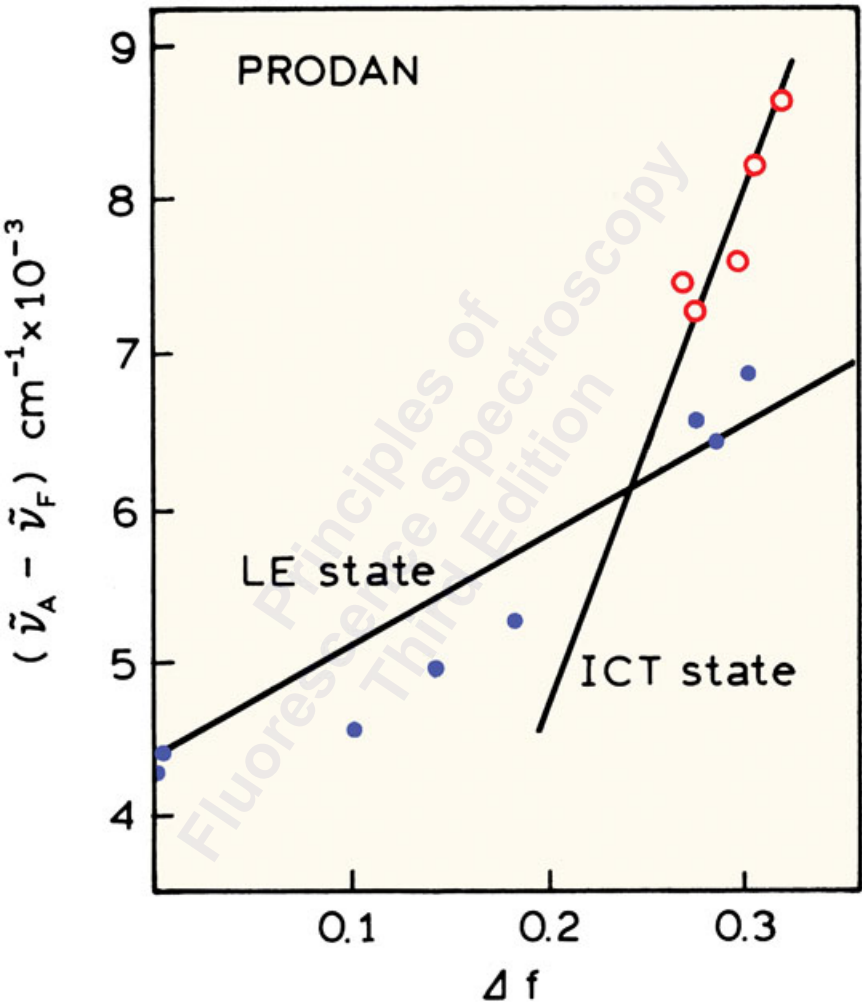


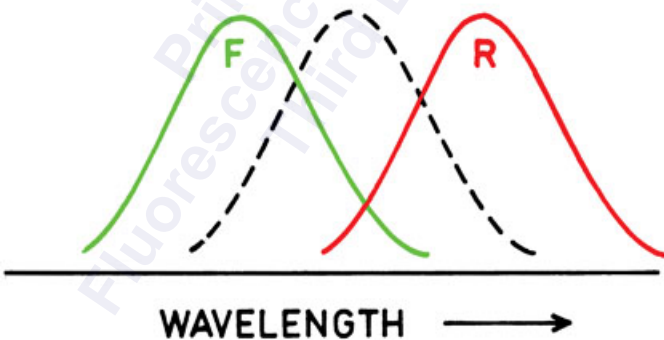
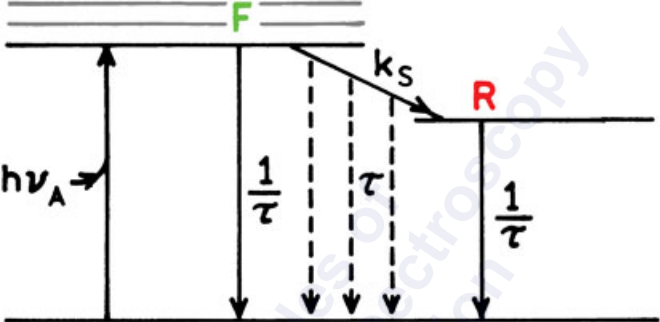


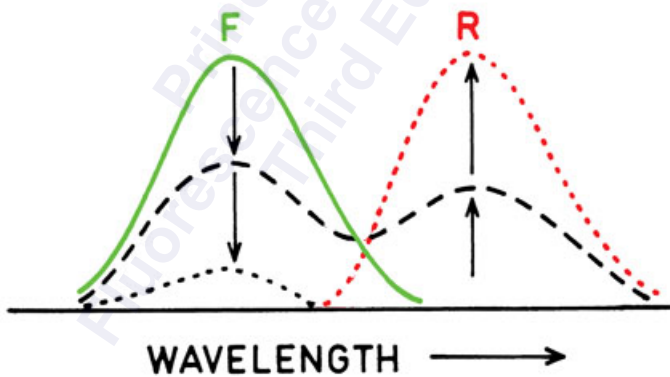
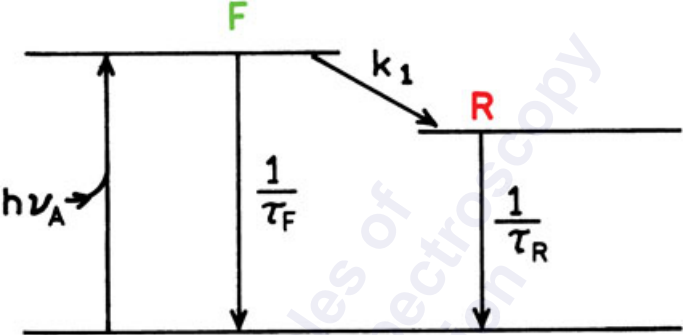


Alpha (Tau)

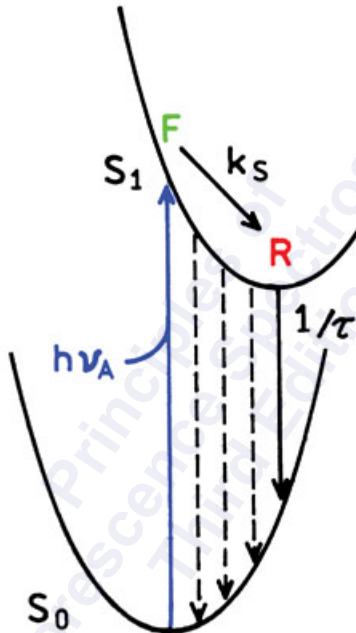








Energy



Reaction Coordinate

Energy

$S_1^F$

F

$k_1$

$S_1^R$

R

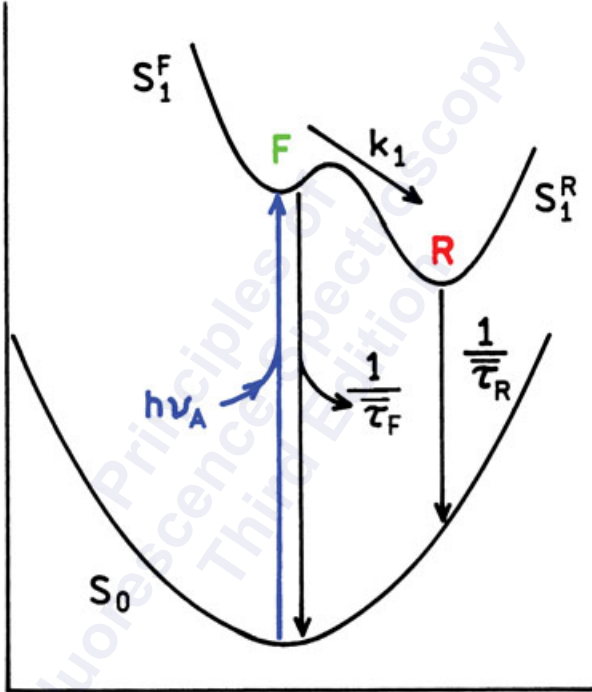
$h\nu_A$

$\frac{1}{\tau_F}$

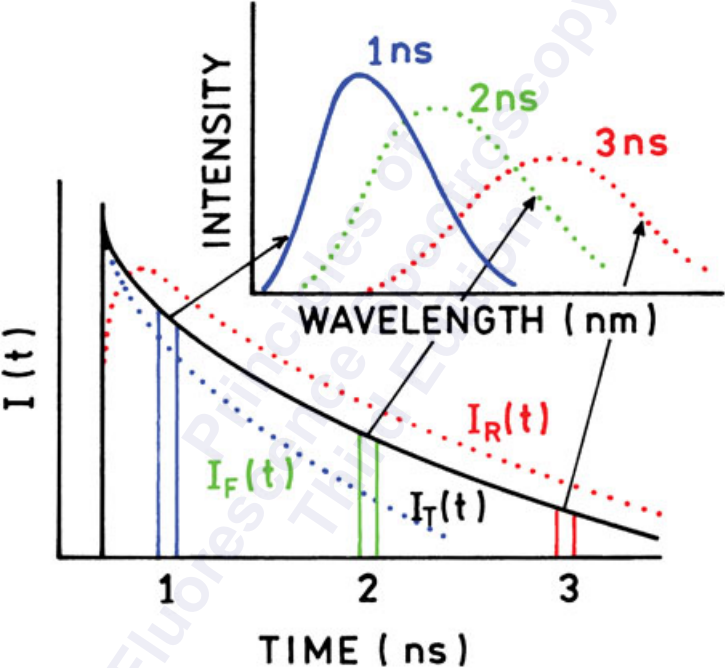
$\frac{1}{\tau_R}$

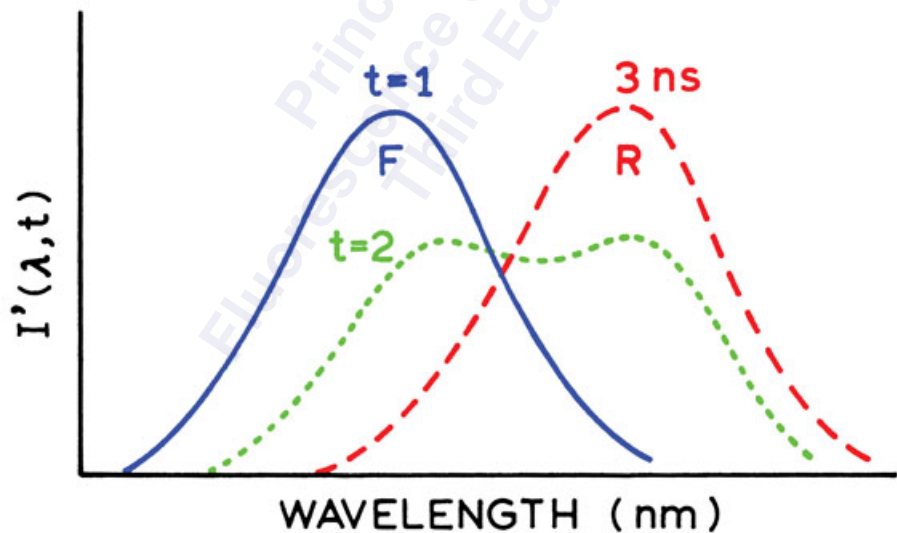
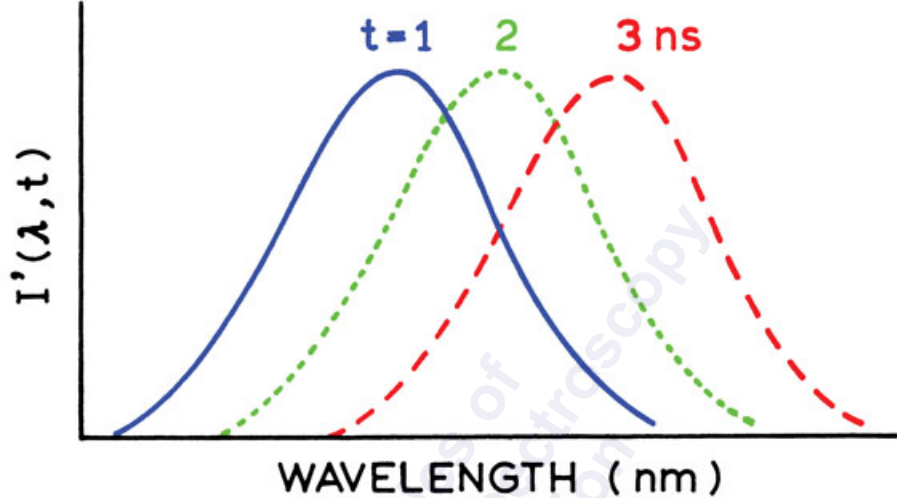
$S_0$

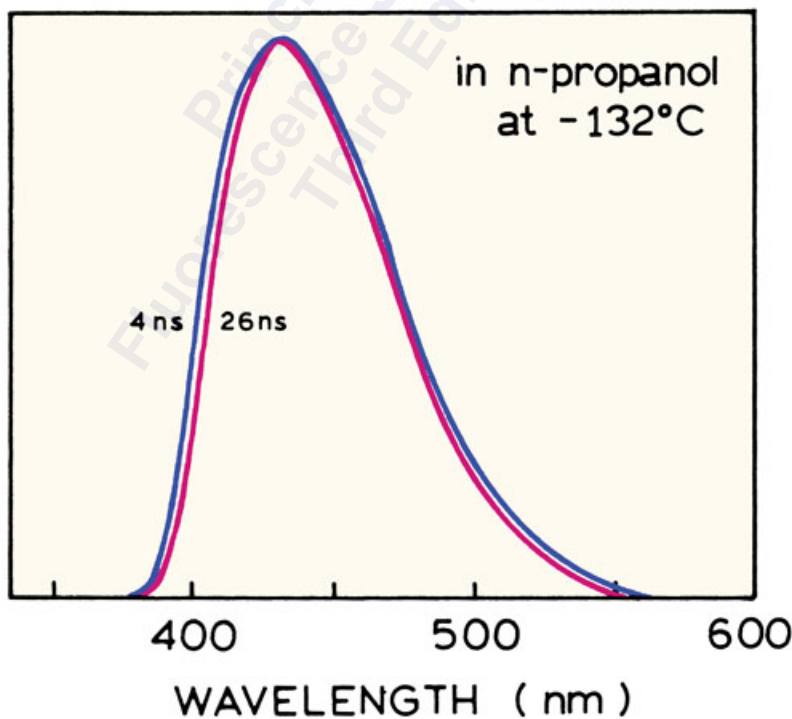
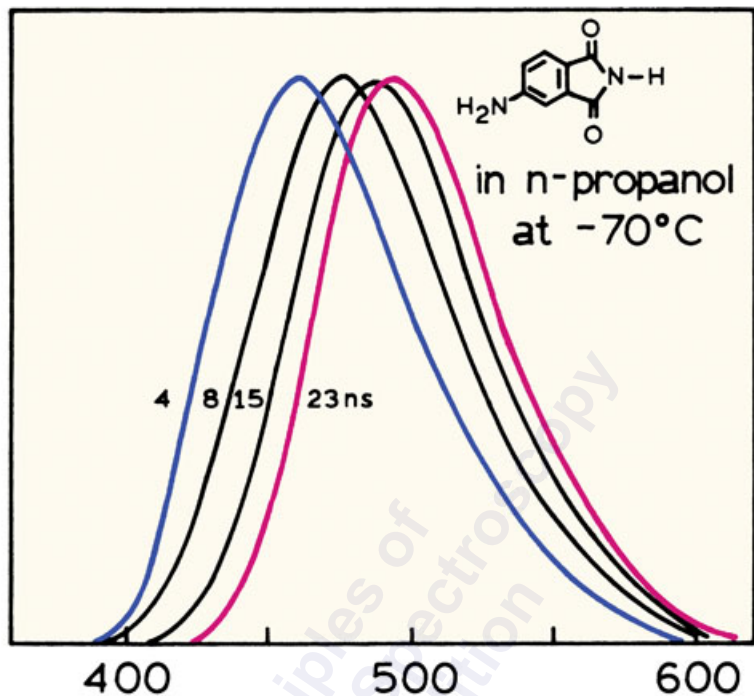
Reaction Coordinate

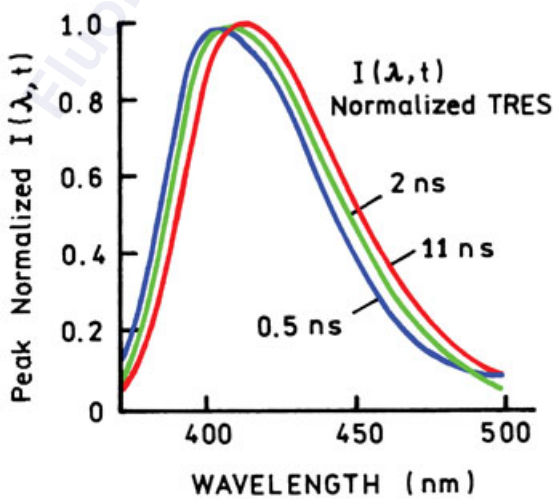
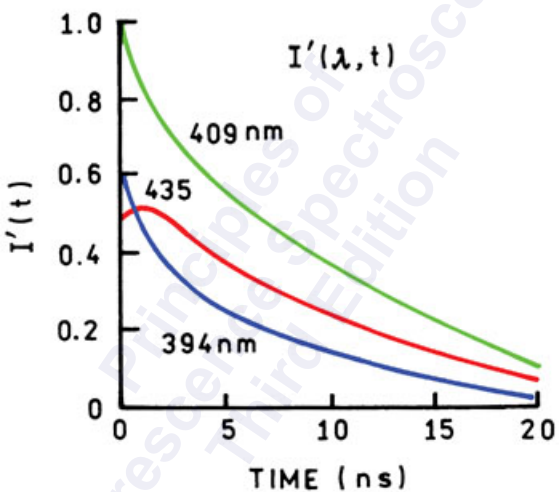
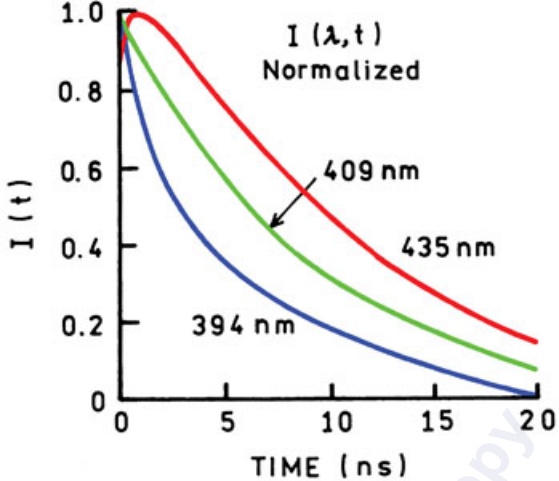




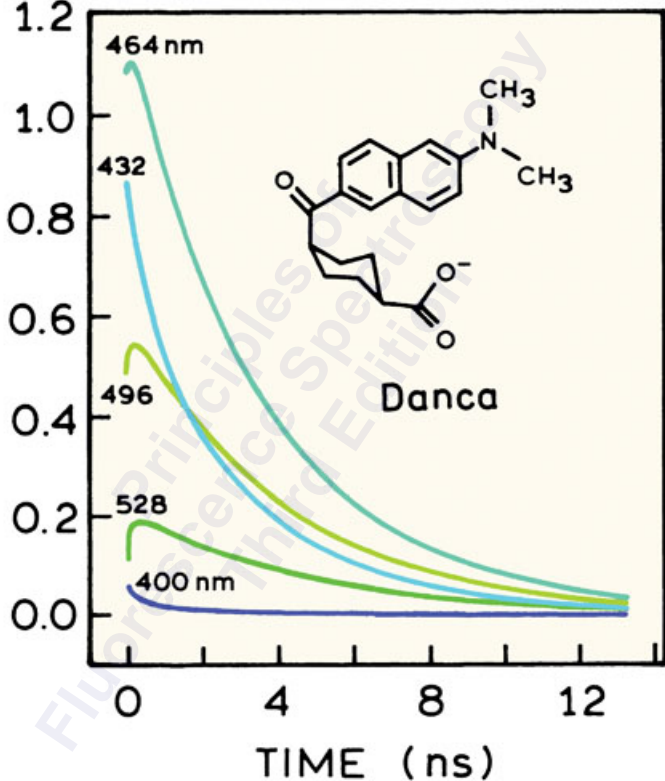








$I'(\lambda, t)$



INTENSITY ( Normalized )

298°K

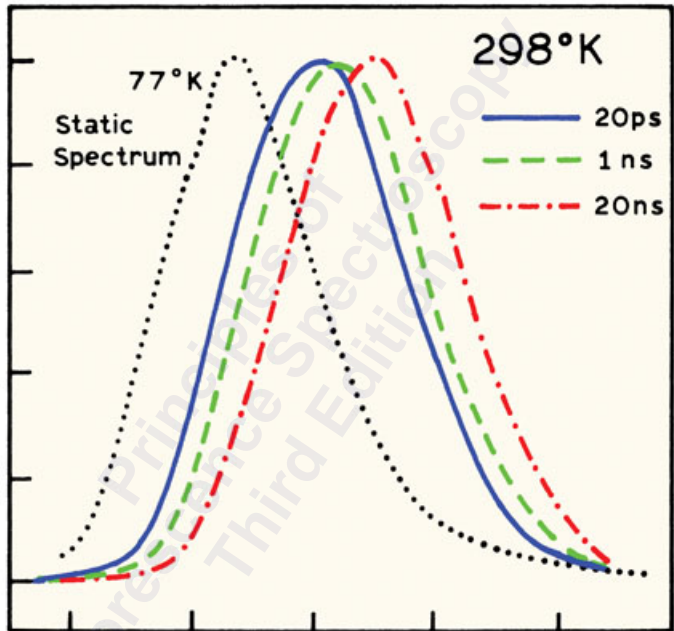
77°K

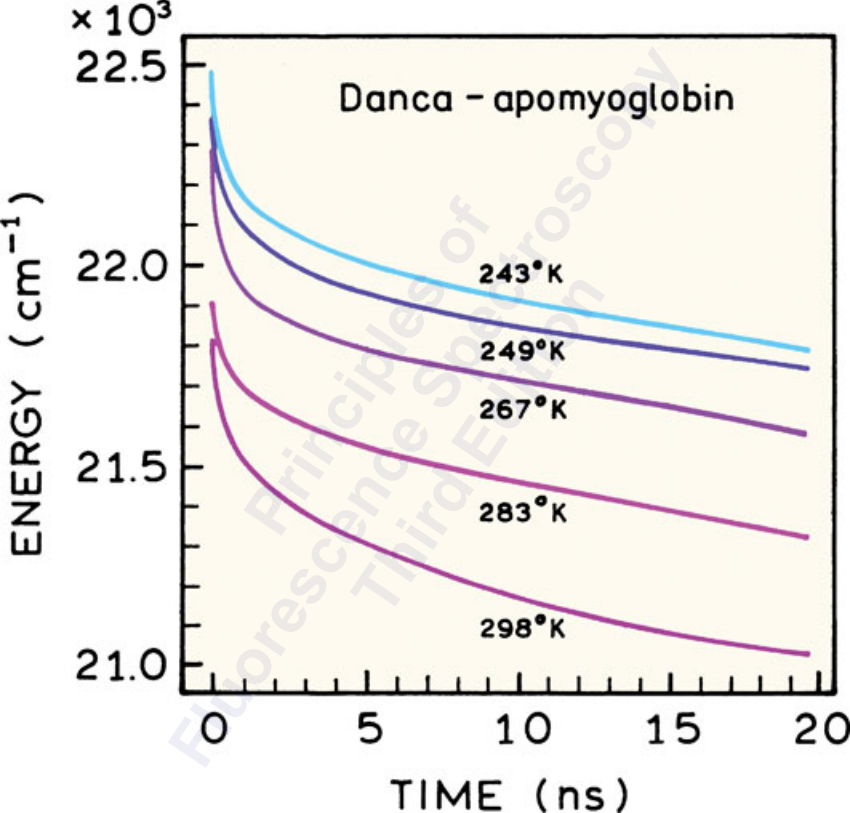
Static  
Spectrum

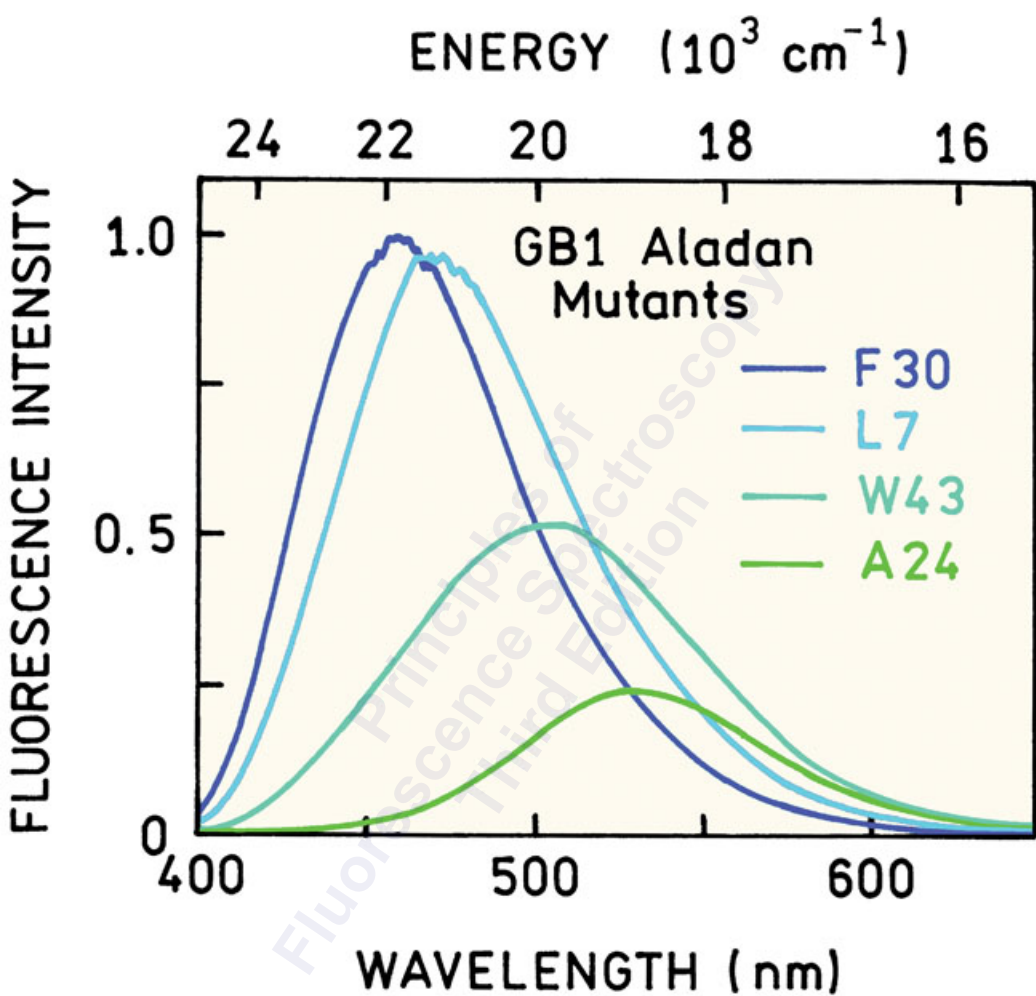
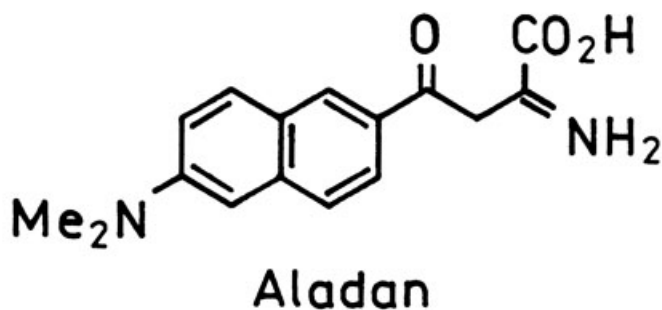
— 20ps  
- - 1 ns  
- · - 20ns

26 24 22 20 18 16

WAVENUMBER ( kK )



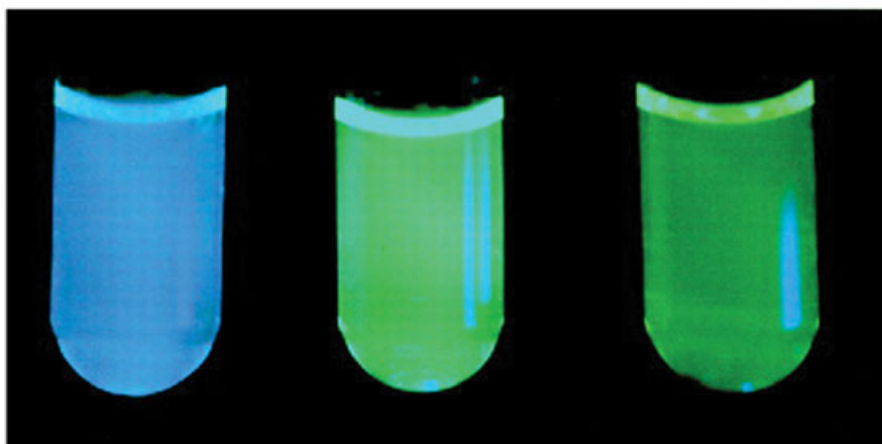




F30

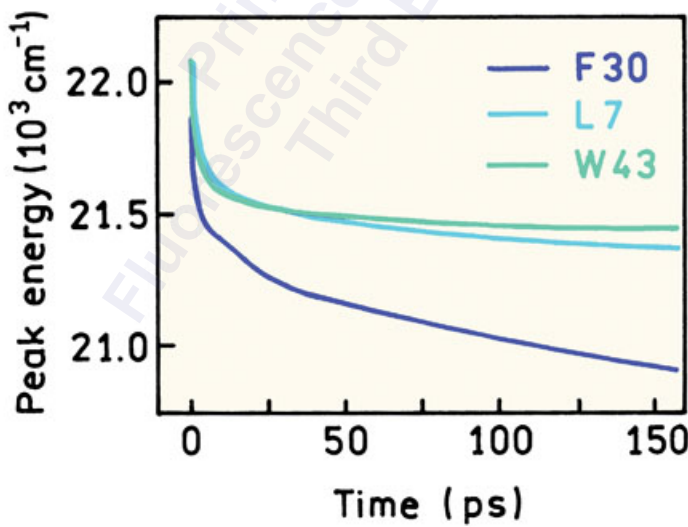
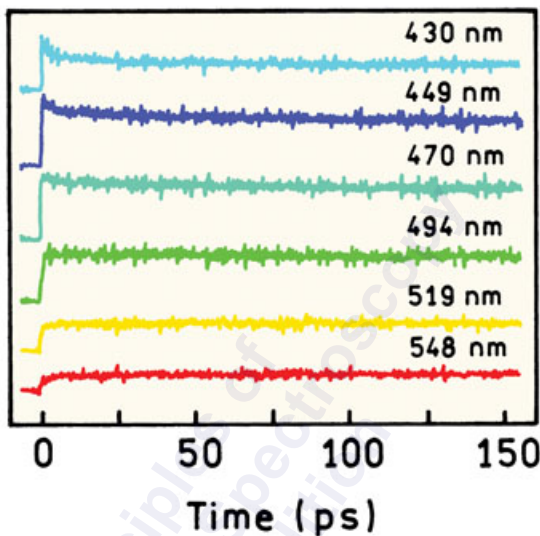
W43

A24

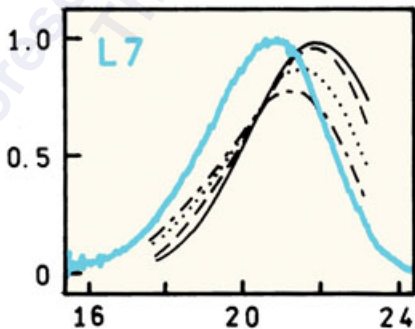
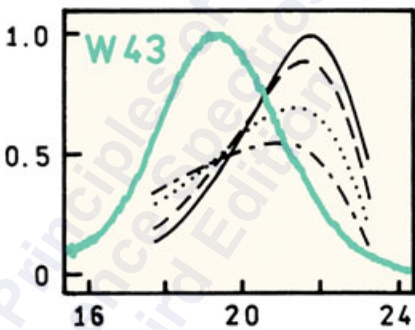
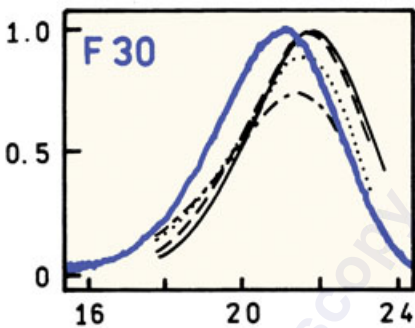




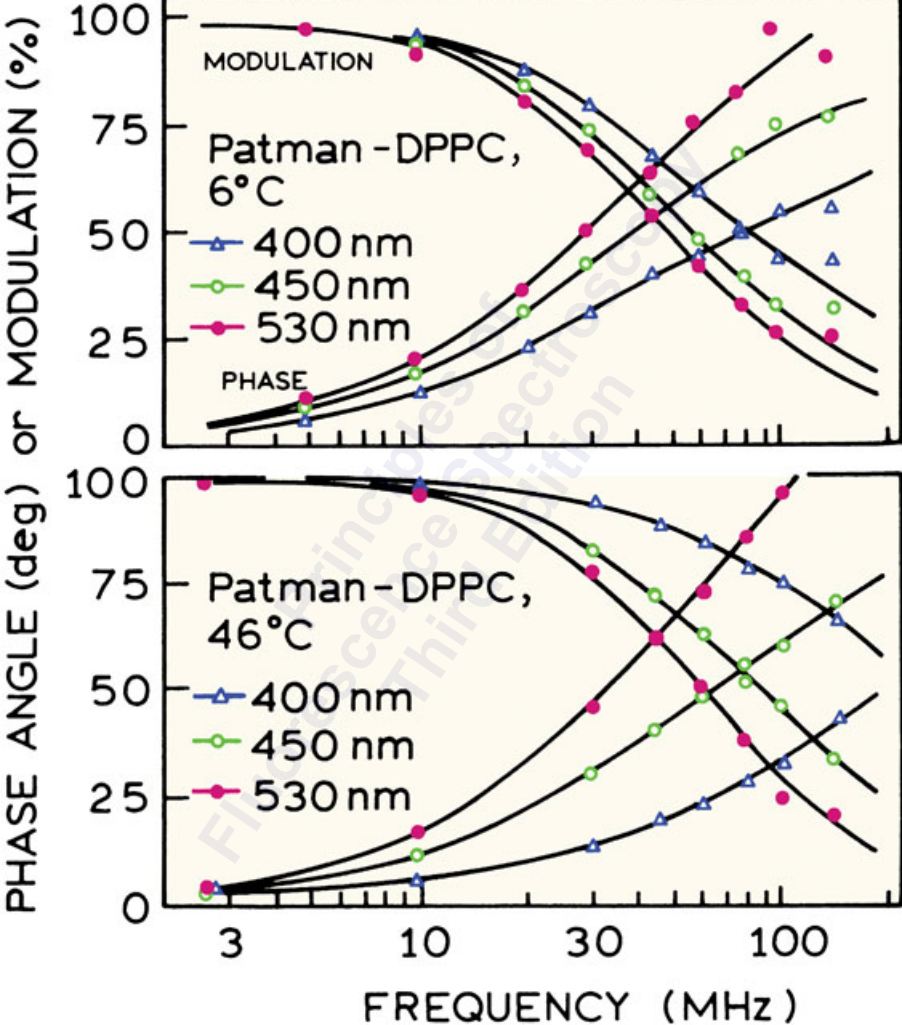
Fluorescence  
Intensity

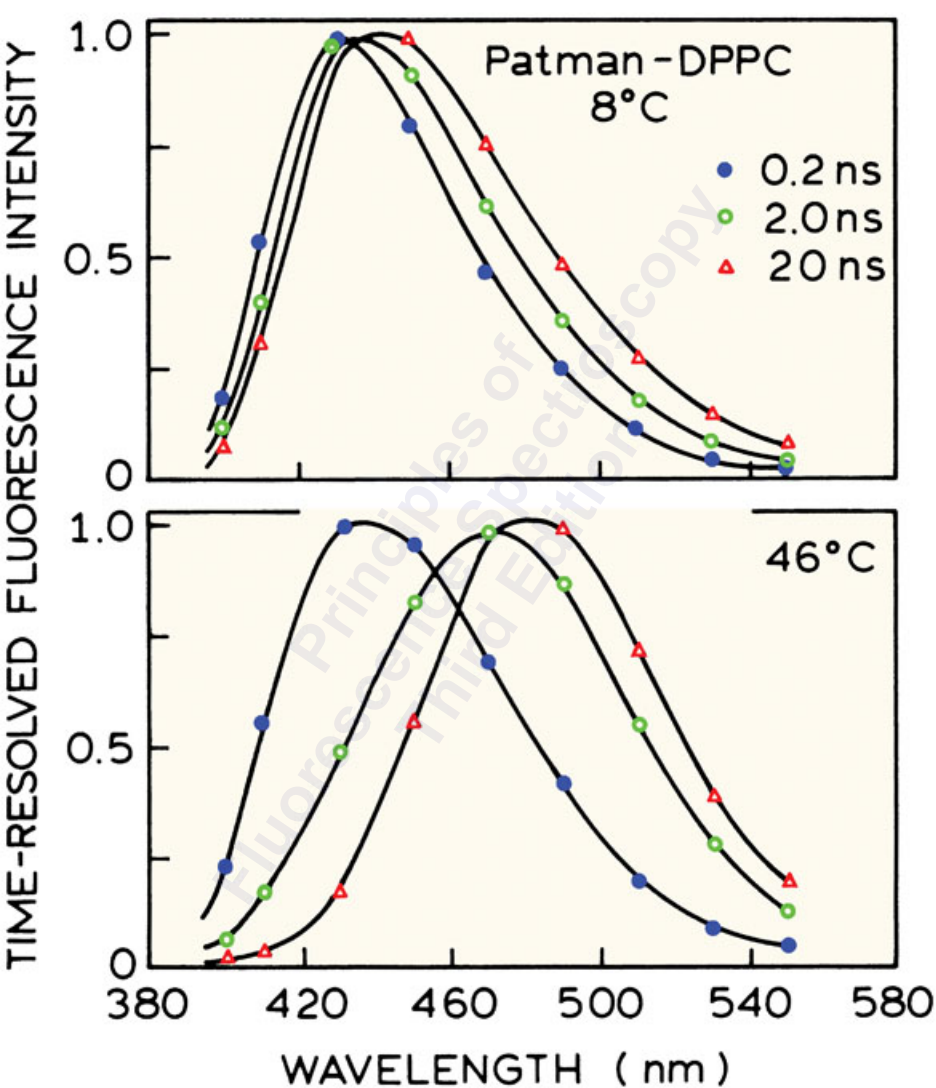


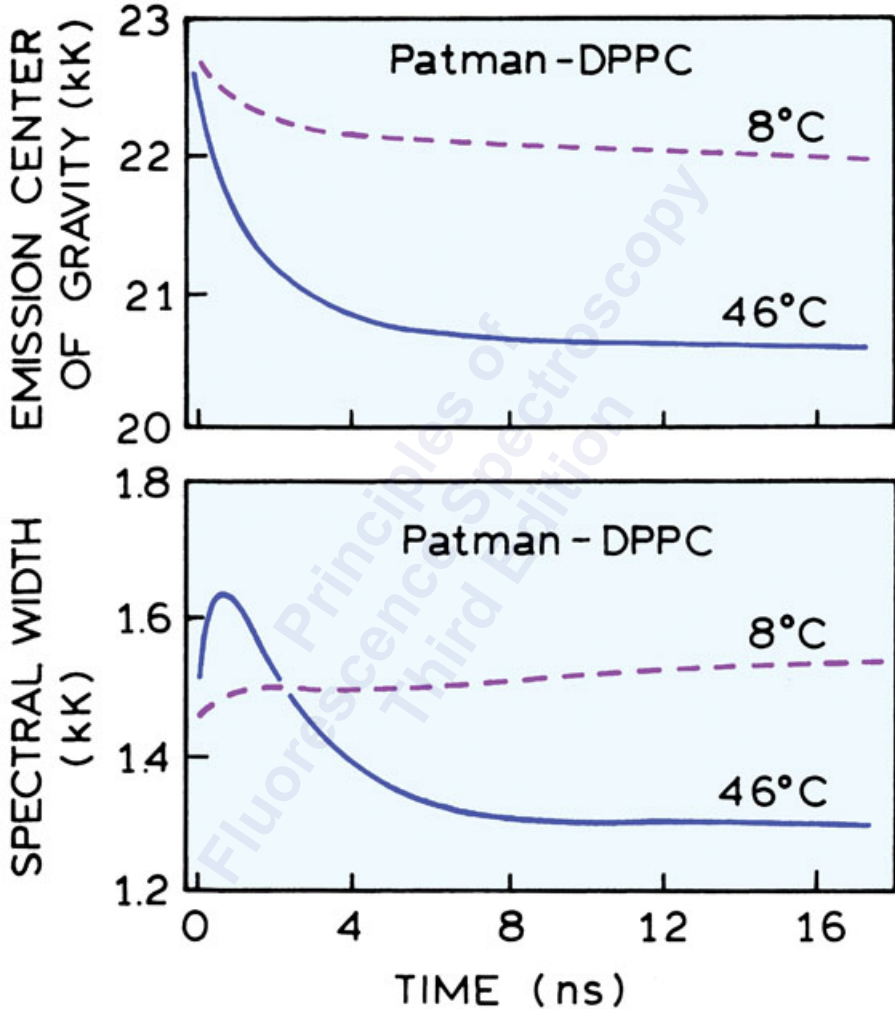
Fluorescence Intensity



Energy ( $10^3 \text{ cm}^{-1}$ )







TIME-RESOLVED

EMISSION MAXIMUM(kK)

23

22

21

5.0

4.5

4.0

0

4

8

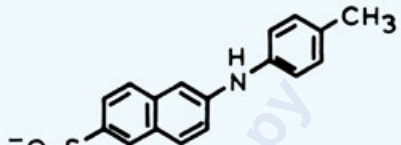
12

16

20

24

TIME (ns)



TNS-labeled  
Egg Lecithin

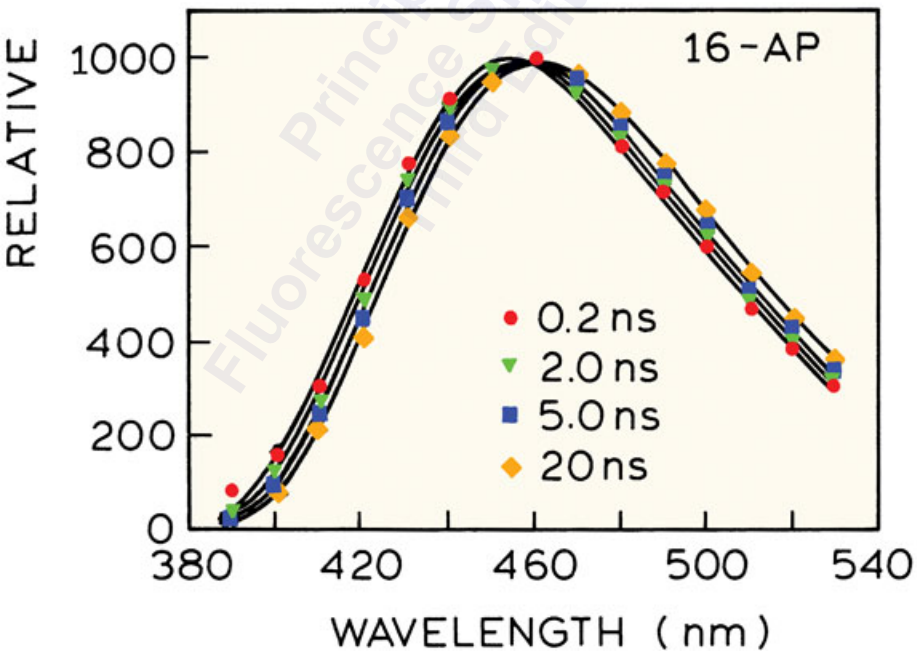
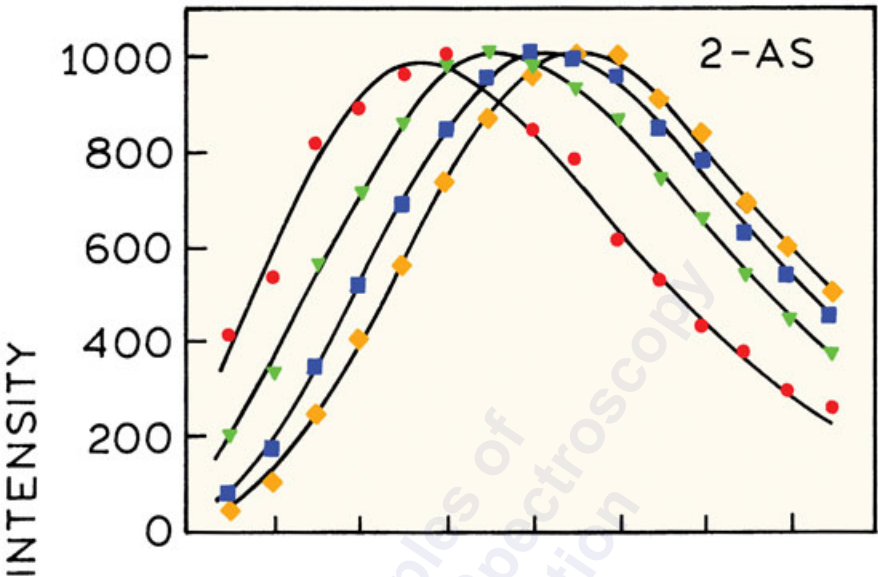
7°C

32°C

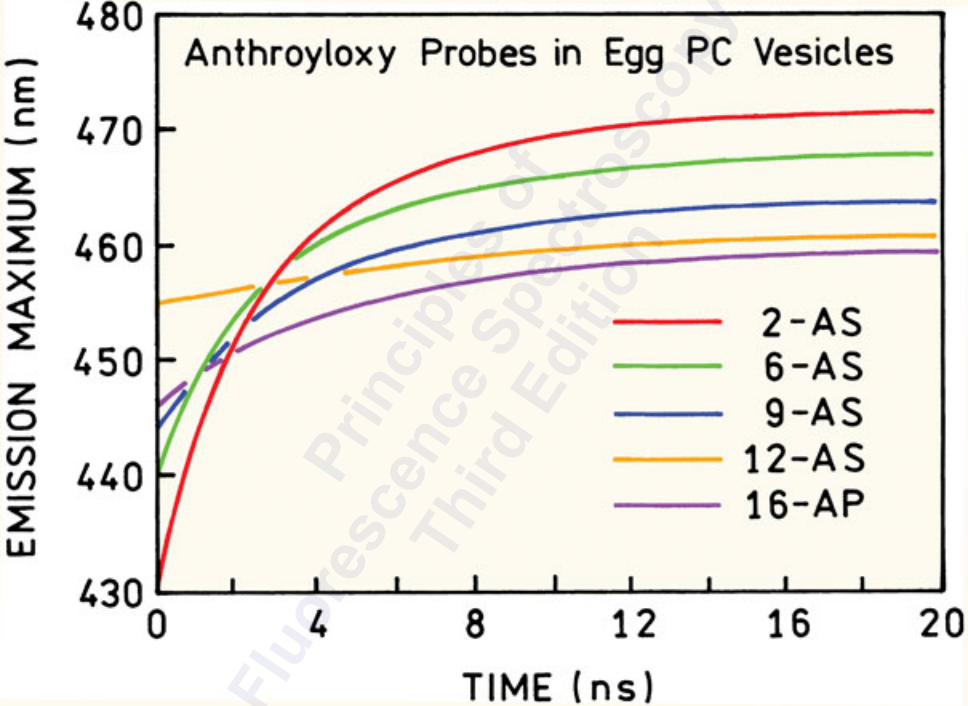
7°C

32°C

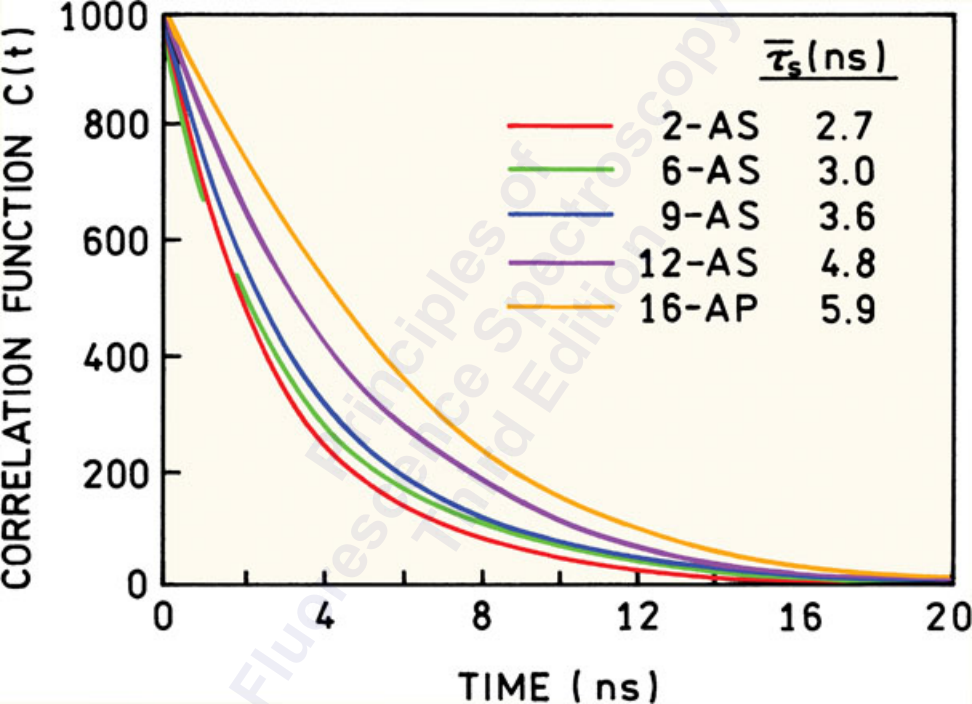
Principles of Fluorescence Spectroscopy  
Third Edition



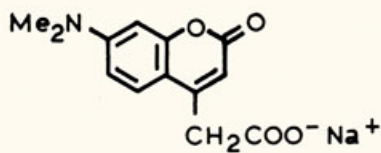
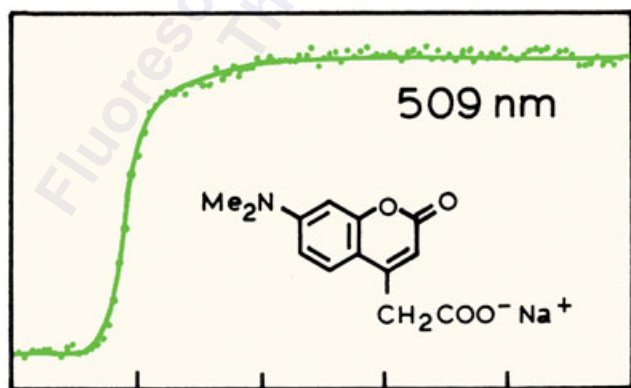
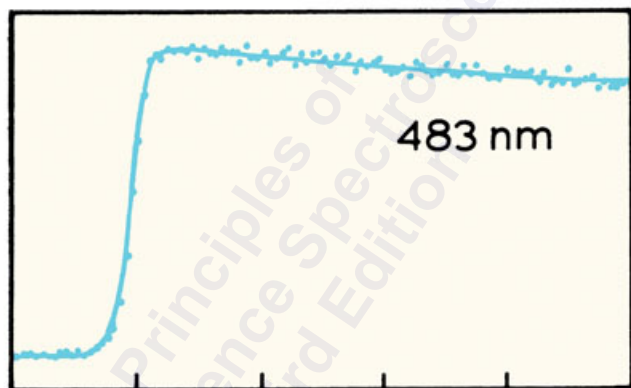
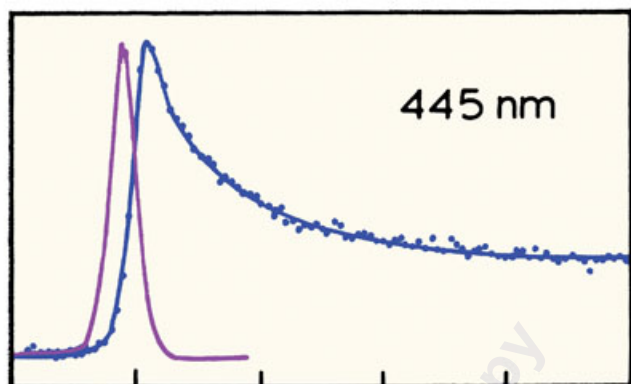






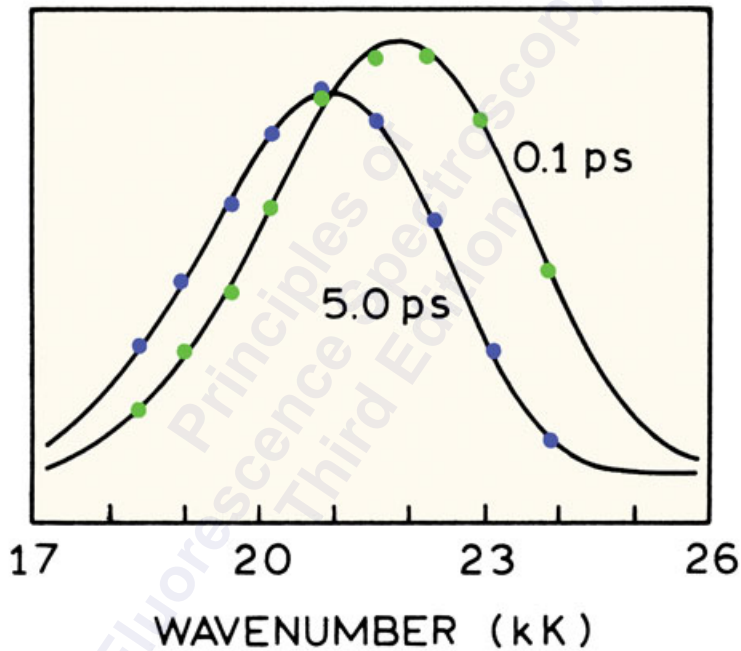


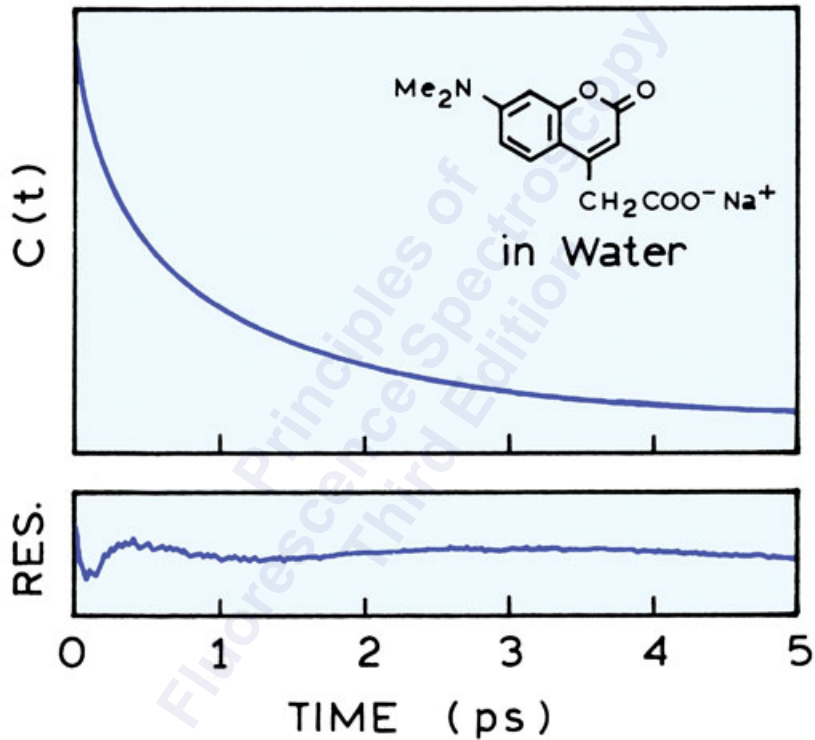
FLUORESCENCE INTENSITY

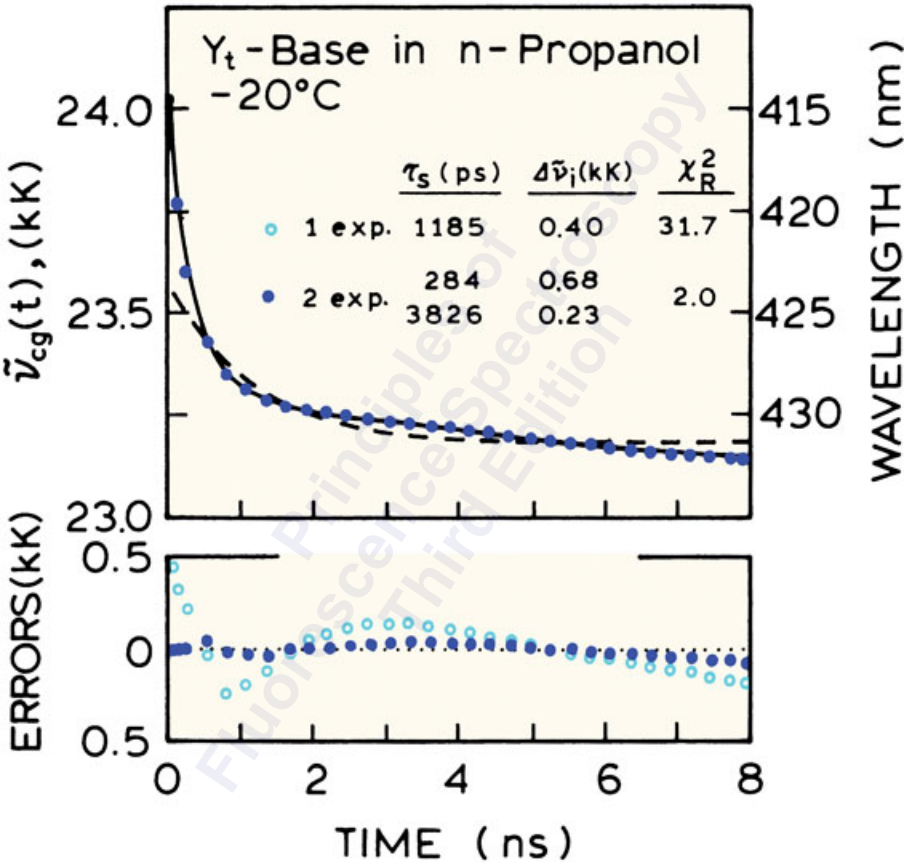


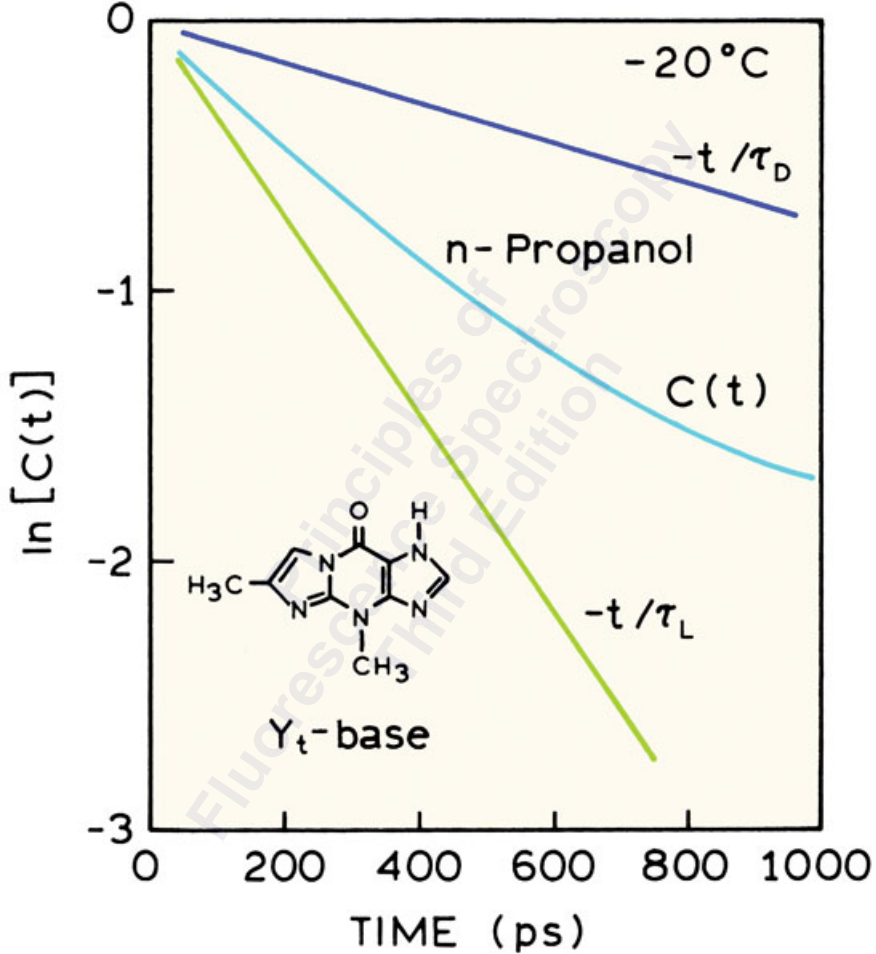
-1 0 1 2 3 4  
TIME (ns)

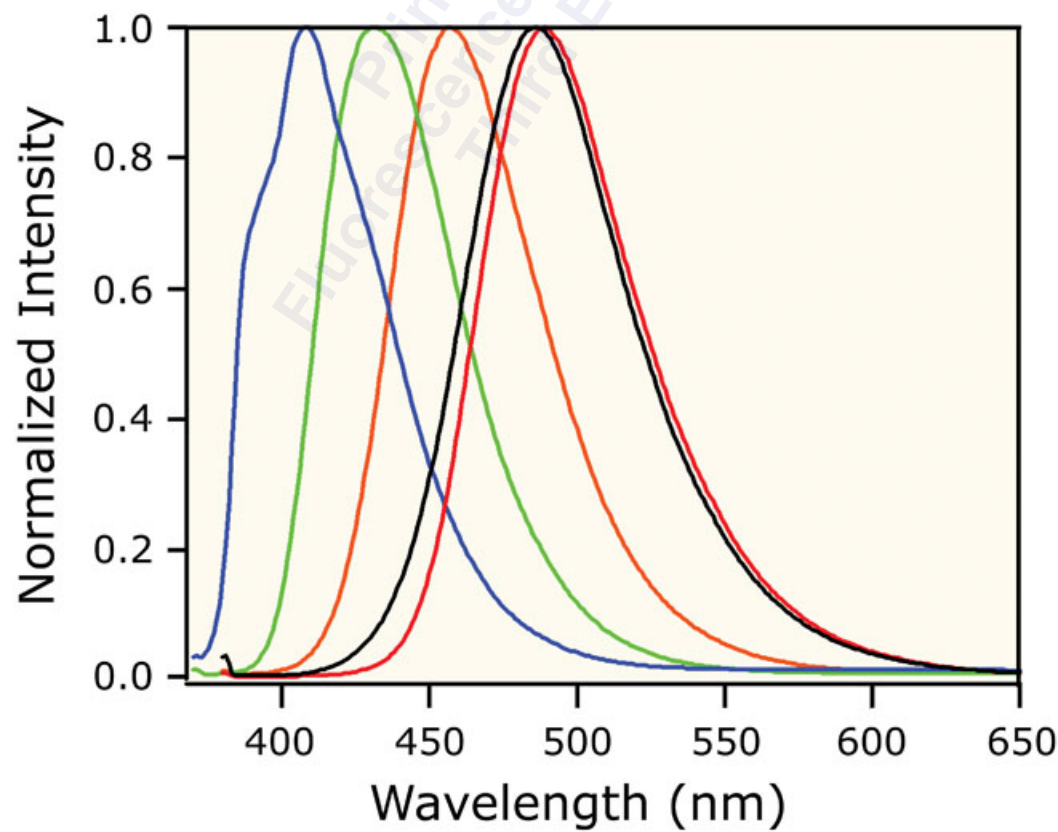
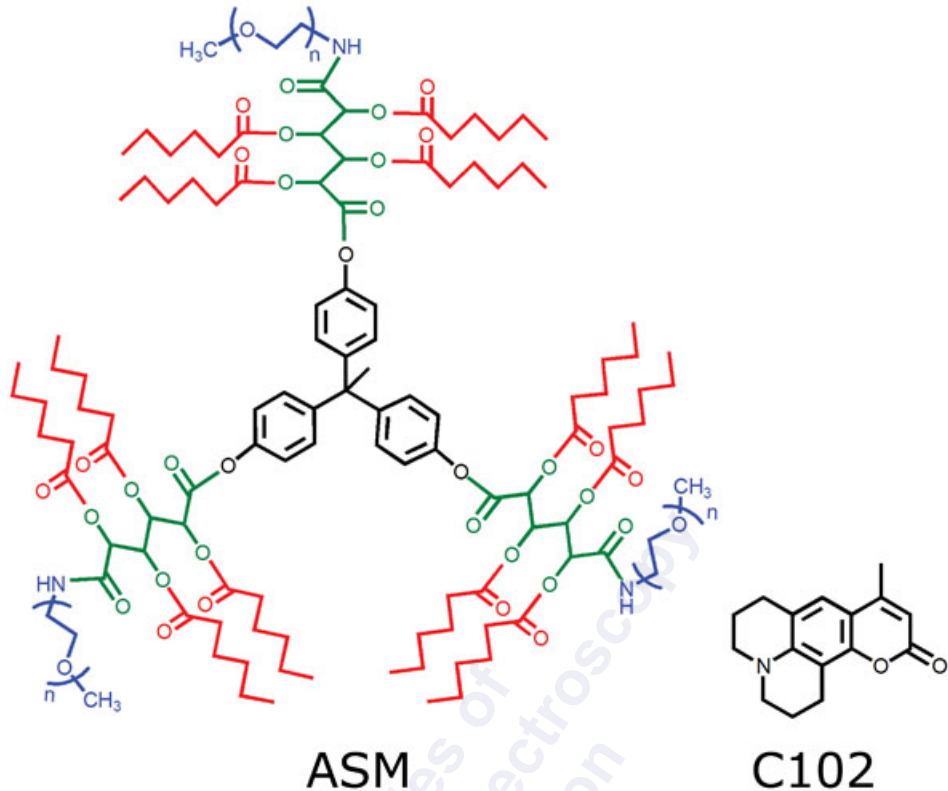
INTENSITY

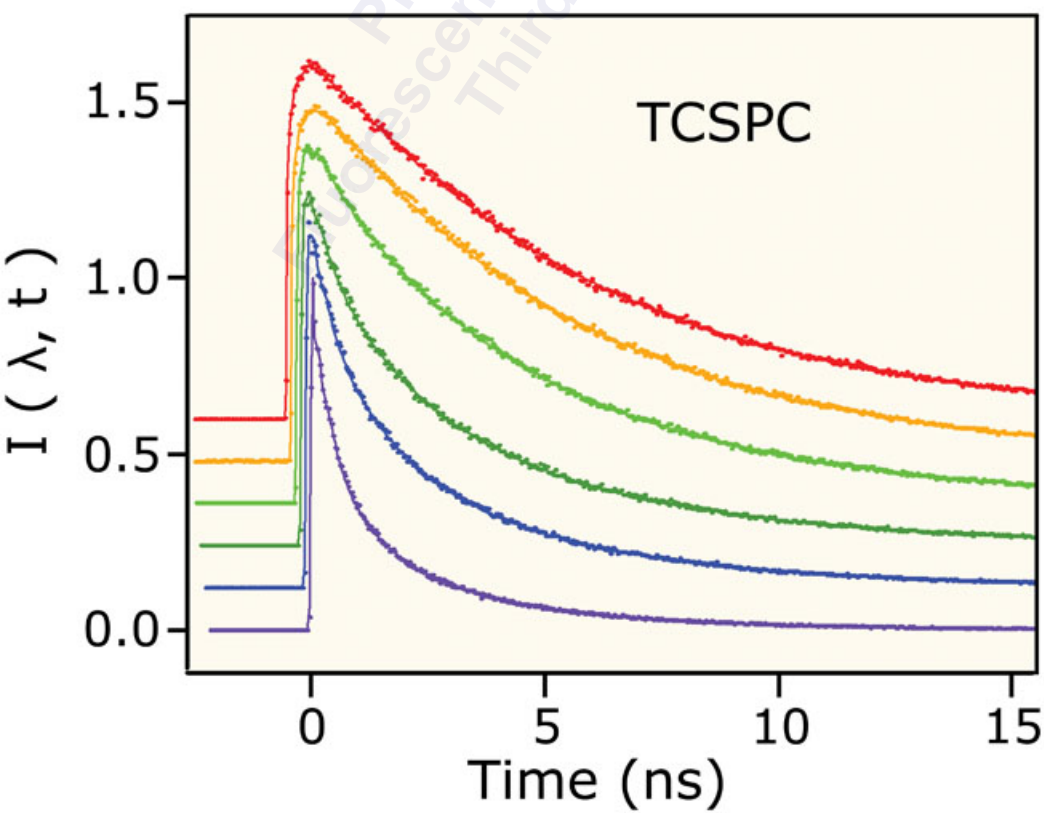
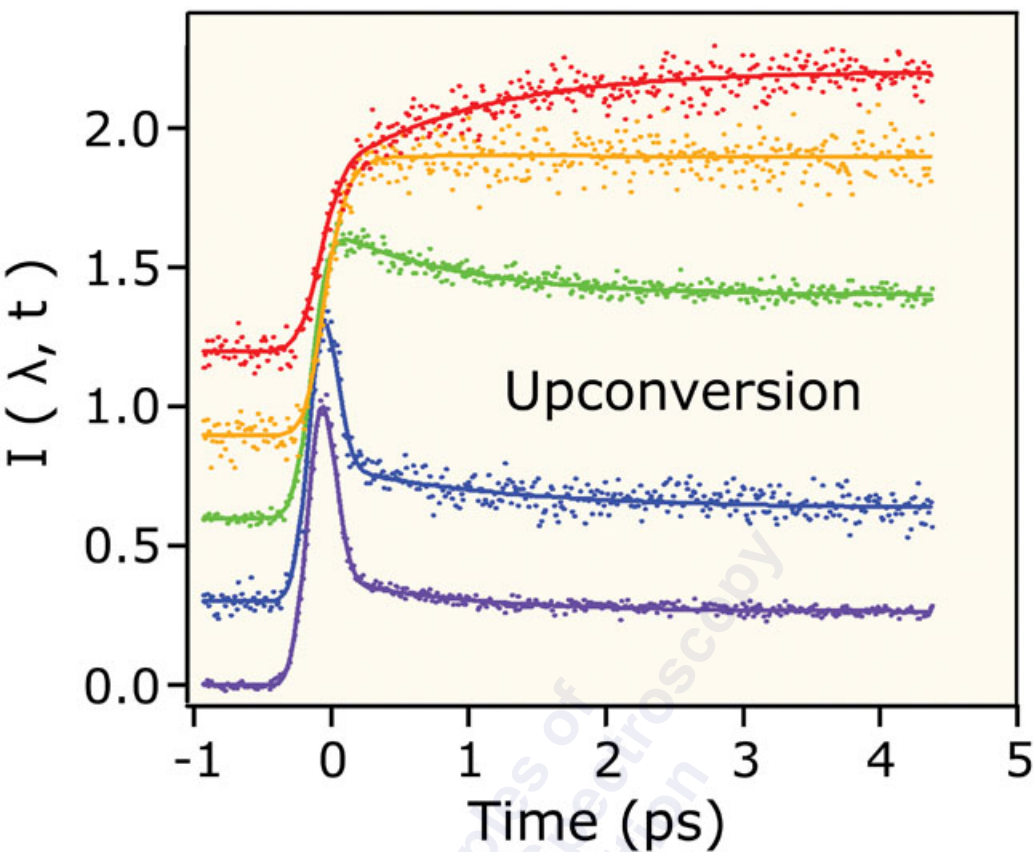




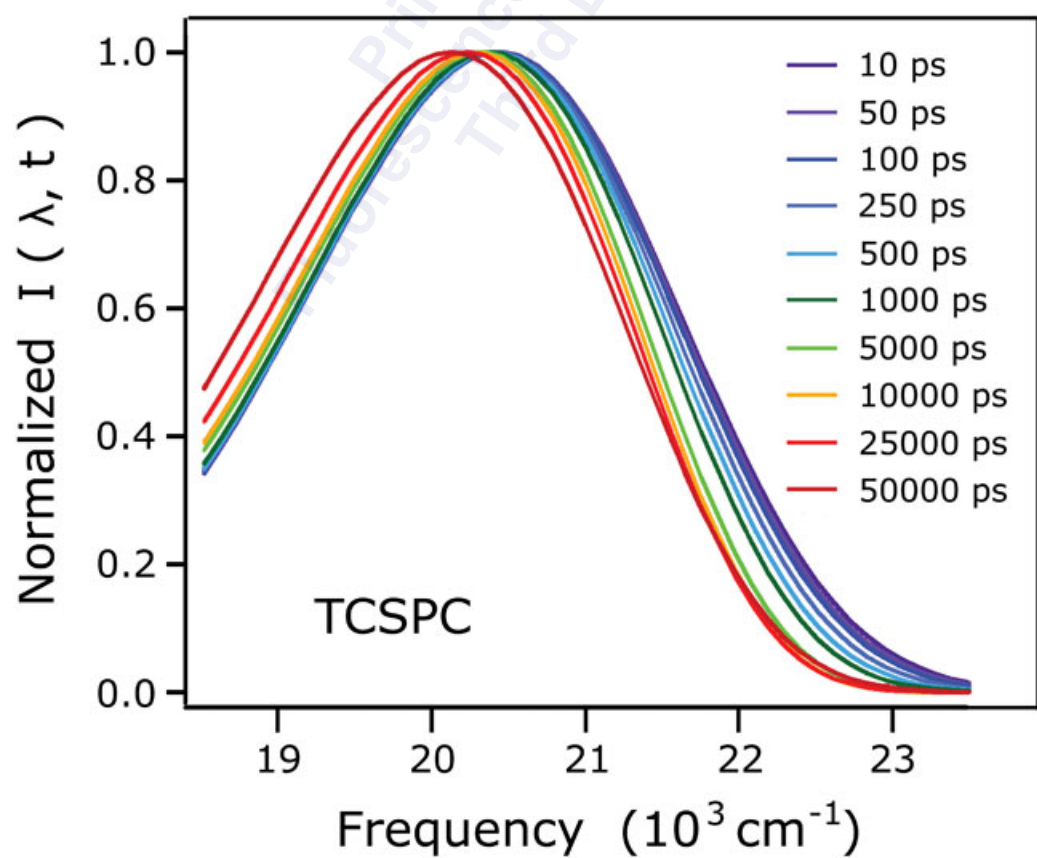
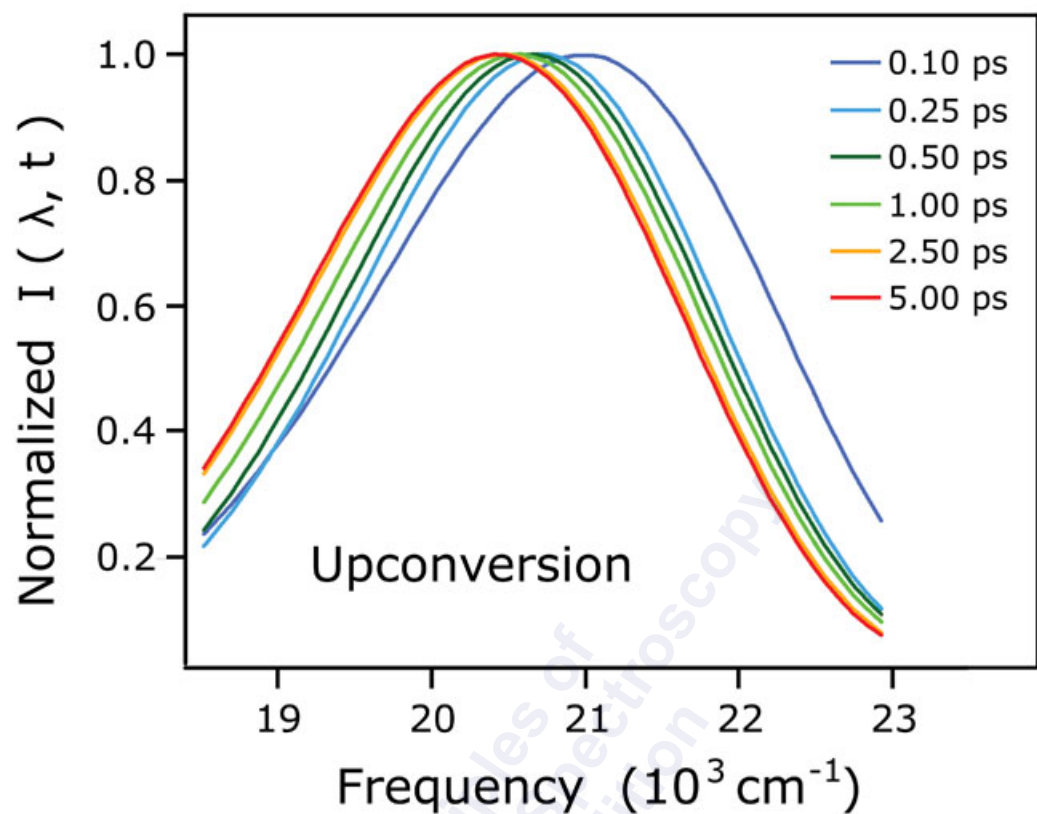




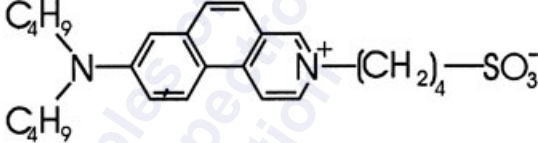




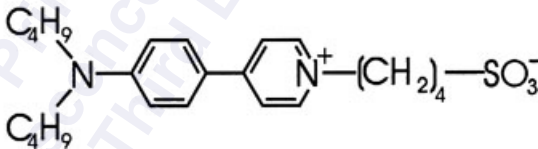


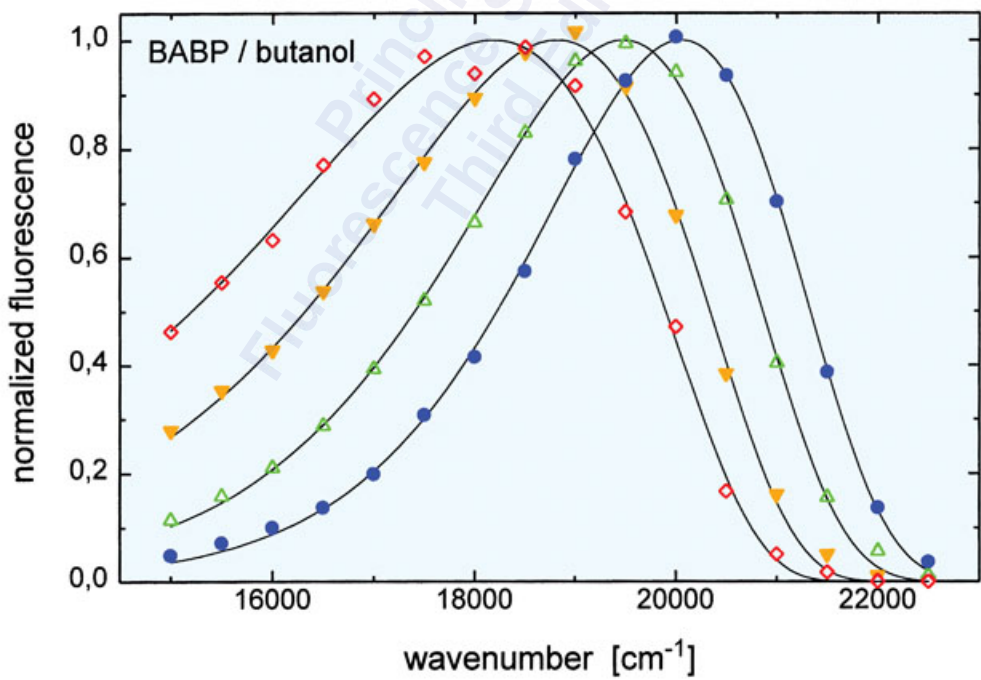
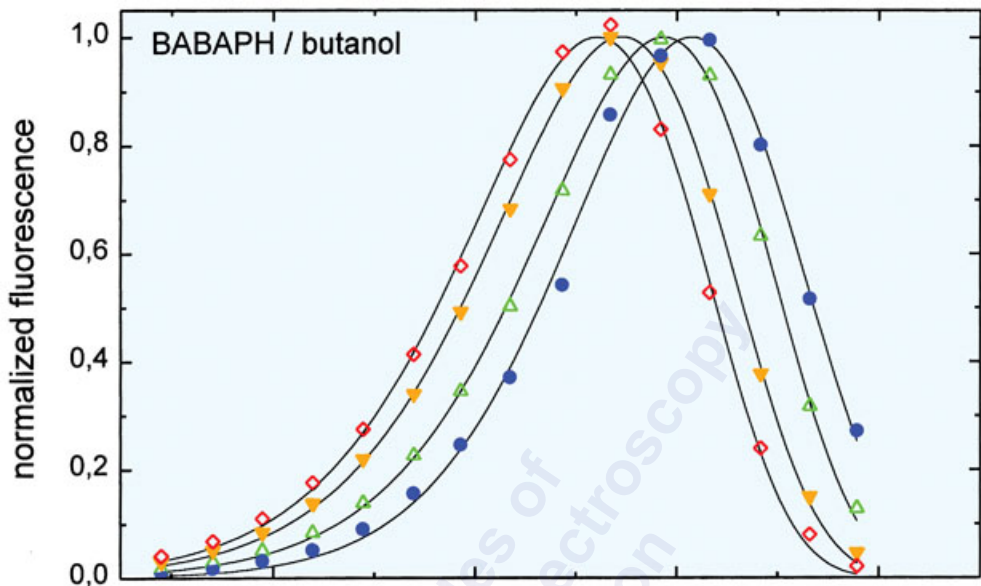


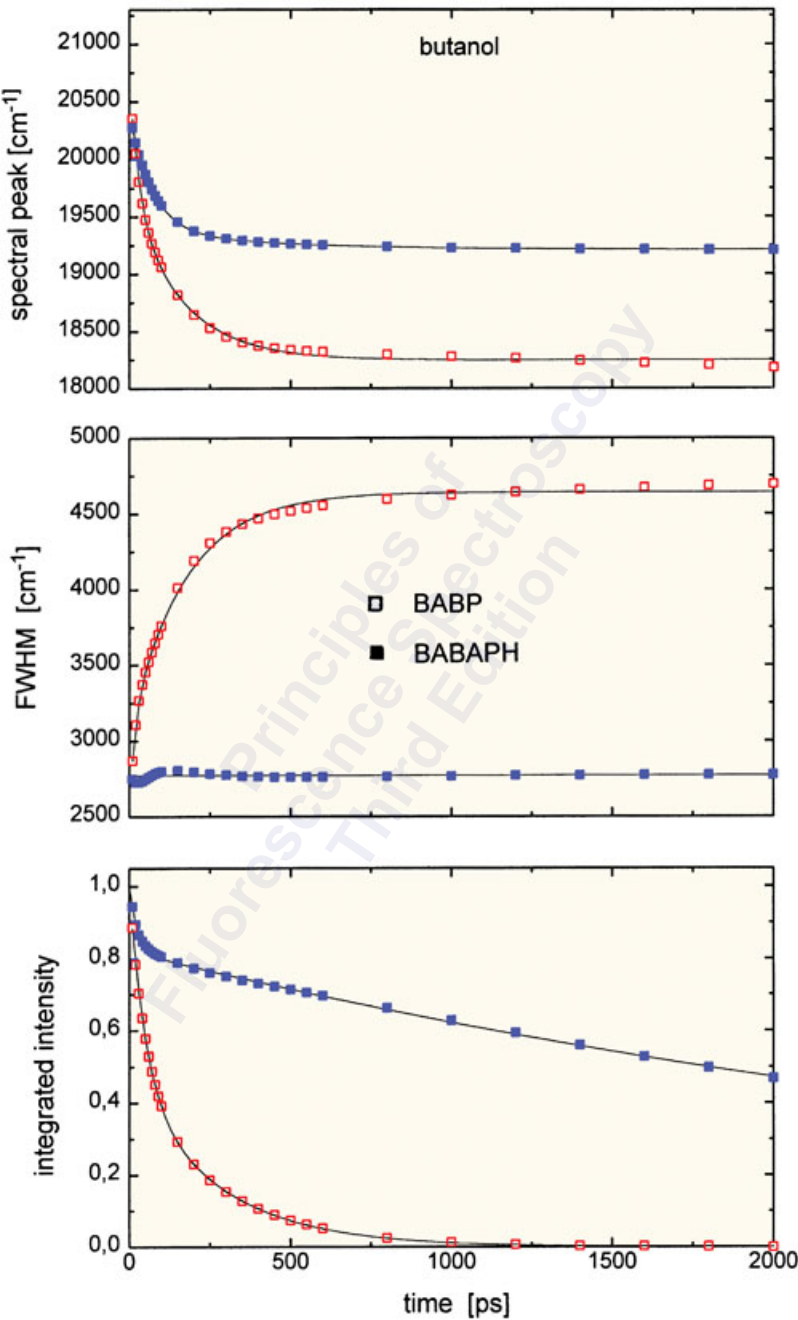
BABAPH



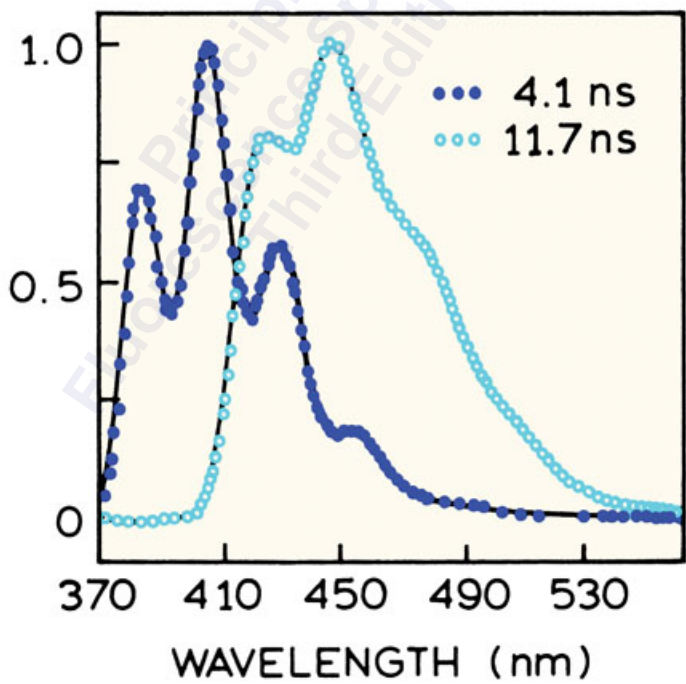
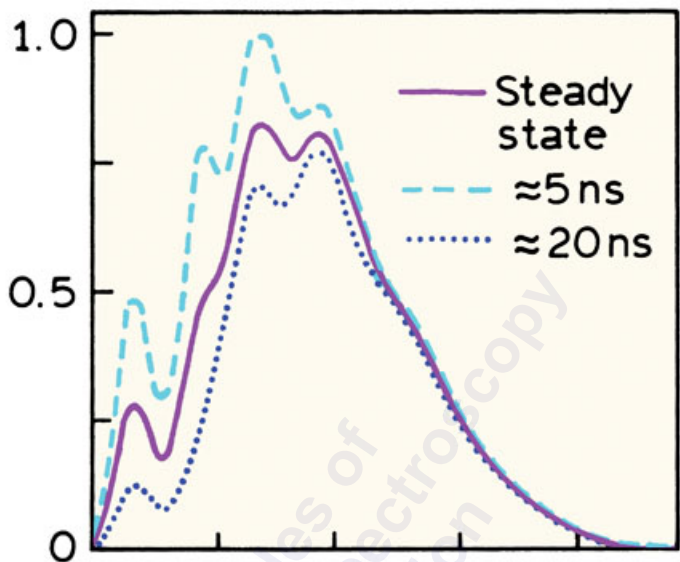
BABP







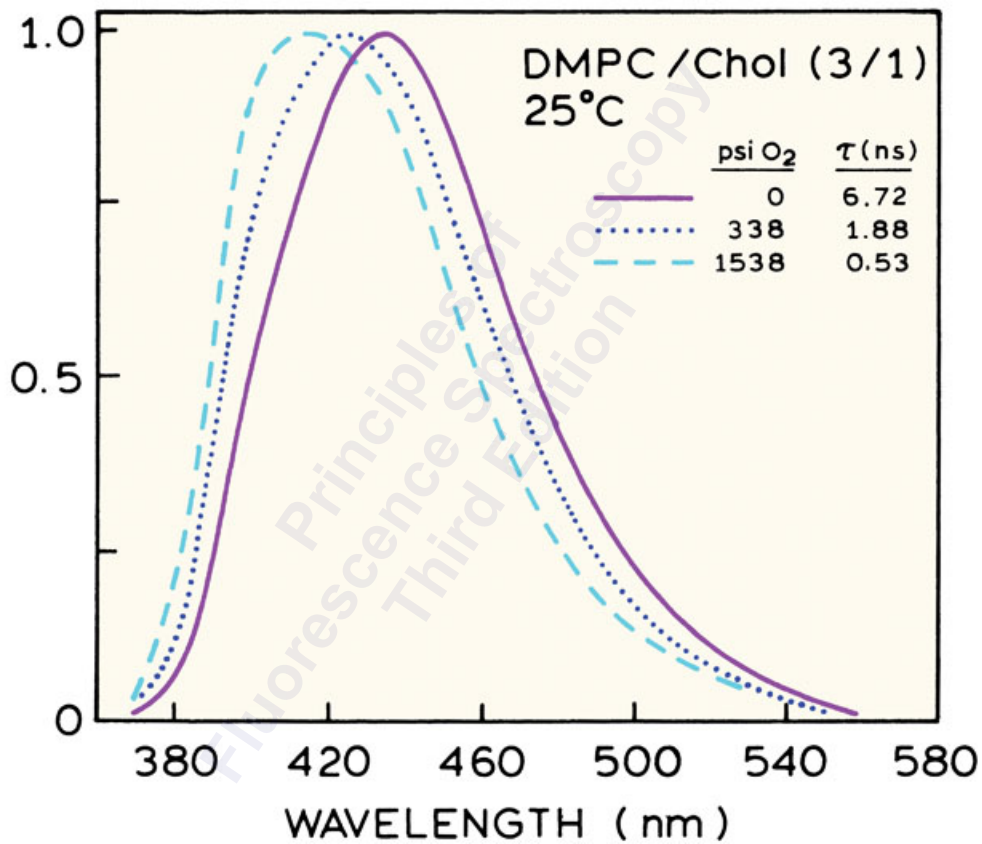
RELATIVE INTENSITY



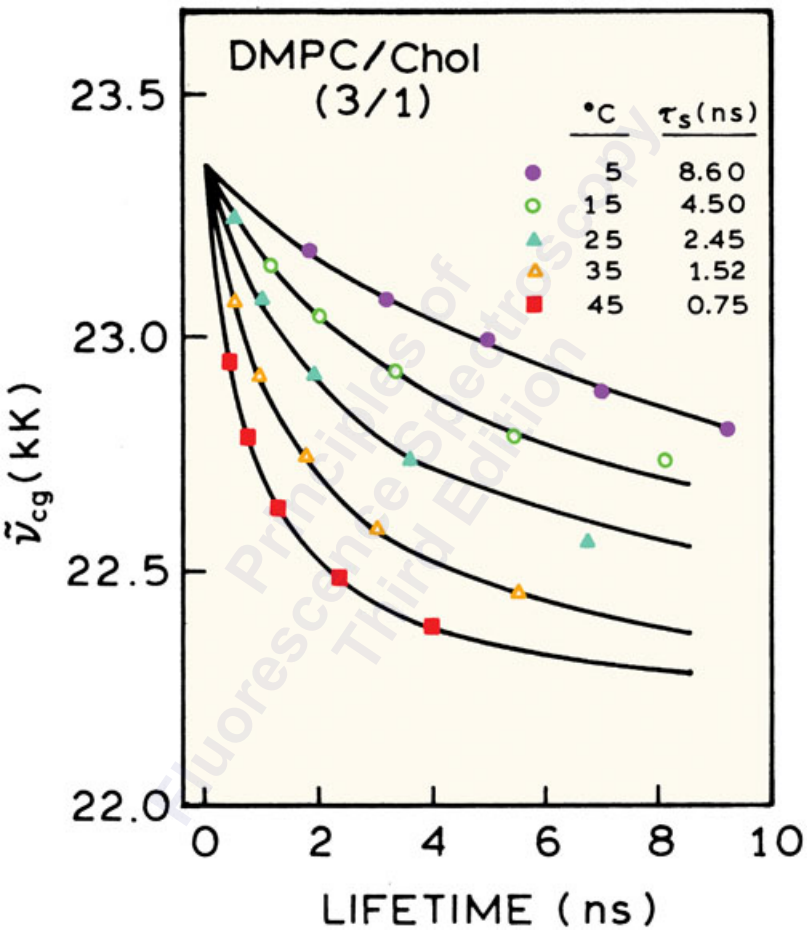
FLUORESCENCE INTENSITY

DMPC / Chol (3 / 1)  
25°C

<u>psi O<sub>2</sub></u>	<u><math>\tau</math> (ns)</u>
0	6.72
338	1.88
1538	0.53



WAVELENGTH ( nm )



INTENSITY

$\lambda_{\text{ex}}(\text{nm})$

— 365

... 405

- - - 436

0

0.5

1.0

24

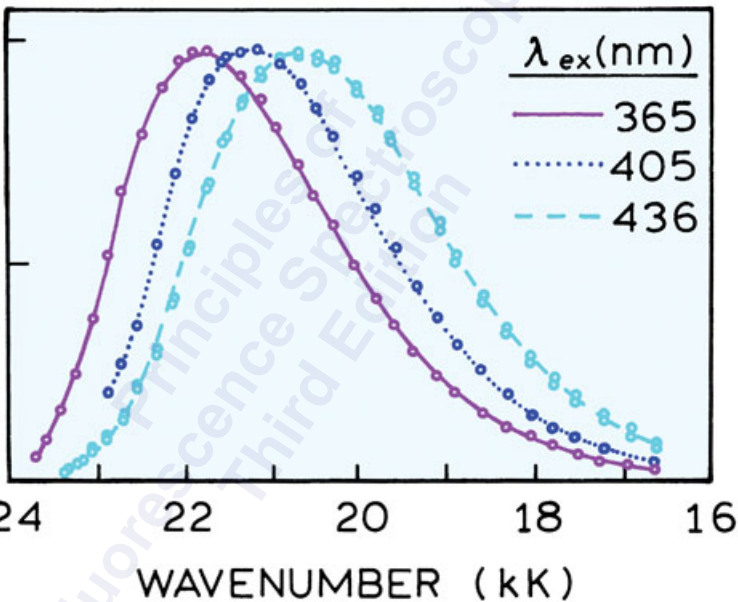
22

20

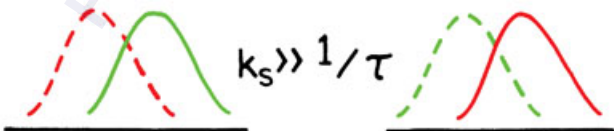
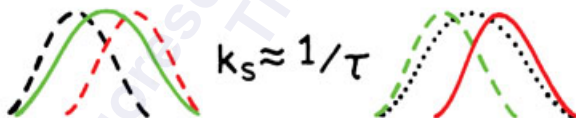
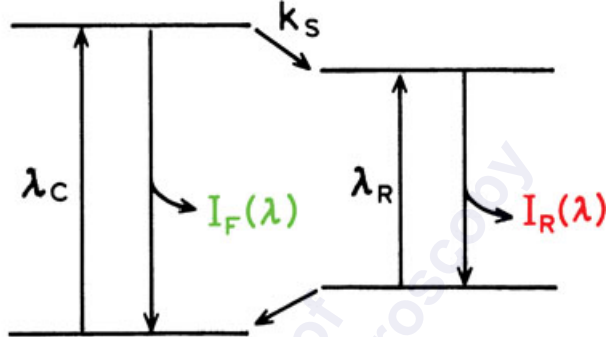
18

16

WAVENUMBER (kK)

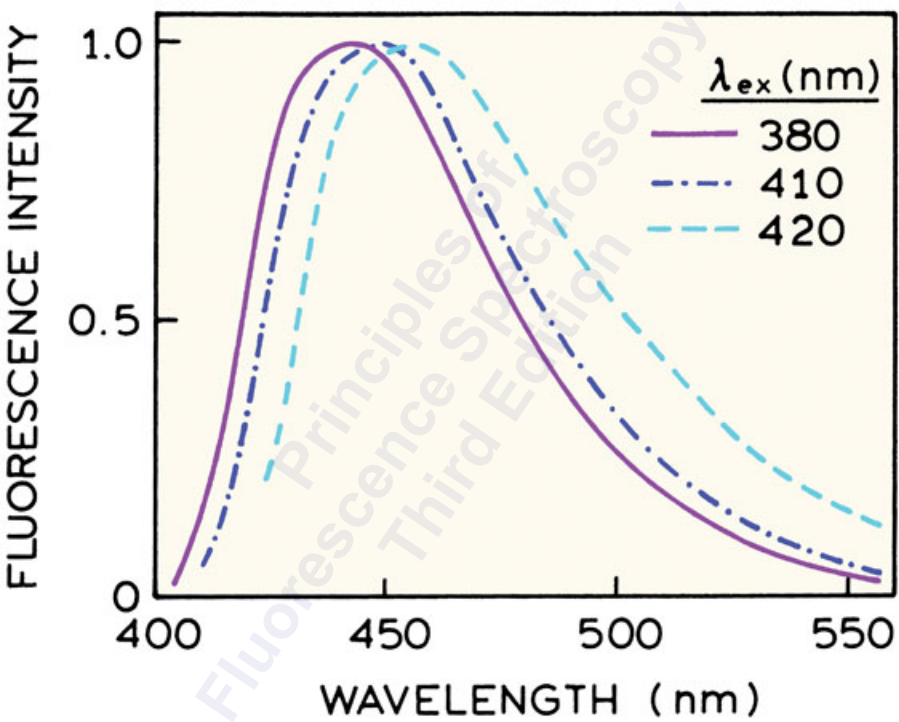


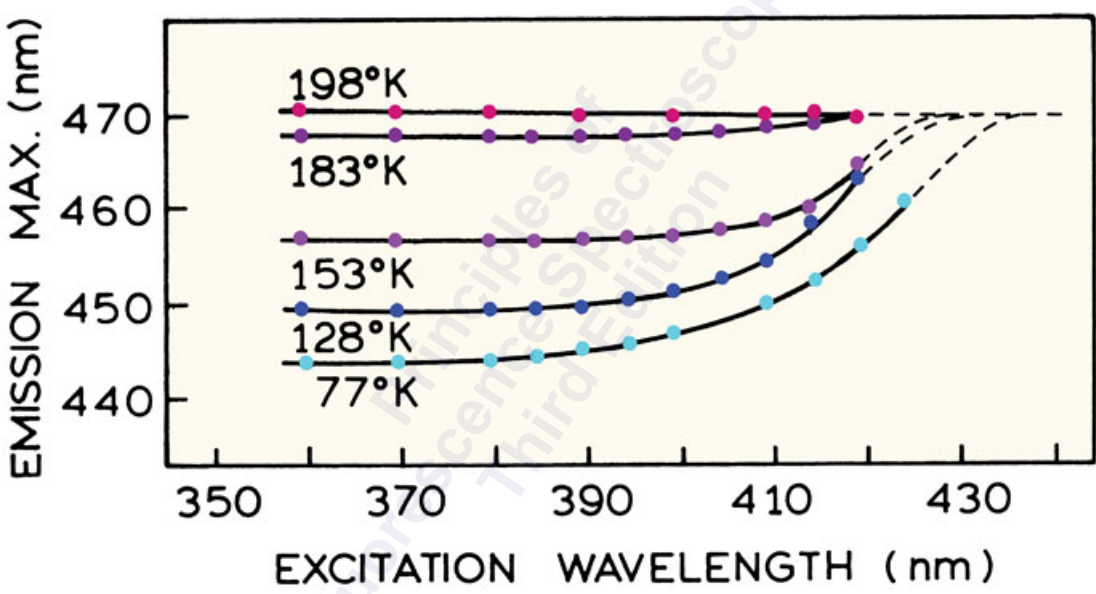


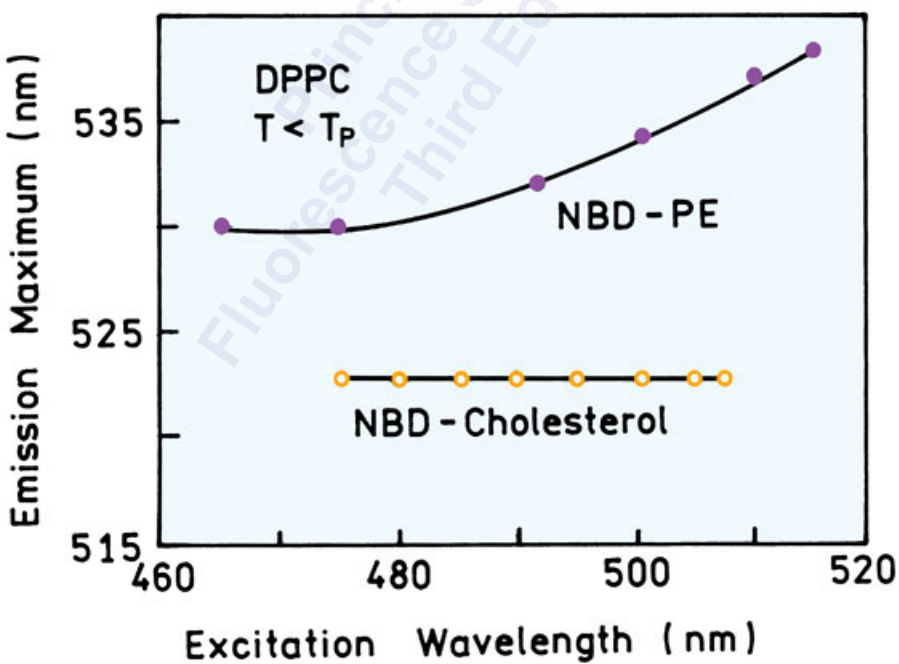
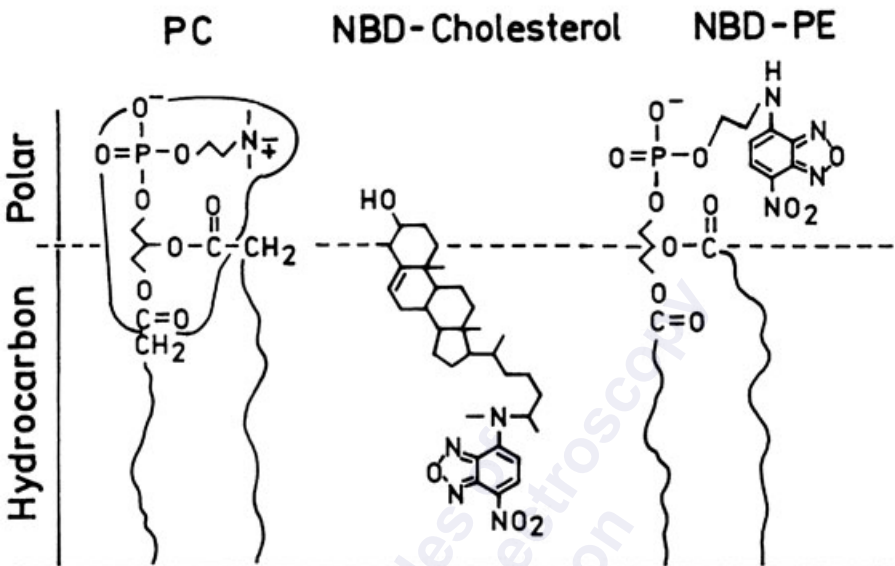


WAVELENGTH

WAVELENGTH







2 - NAPHTHOL

FLUORESCENCE INTENSITY (normalized)

0.1 M HCL

0.05 M NaOH

pH = 3

0

0.5

1.0

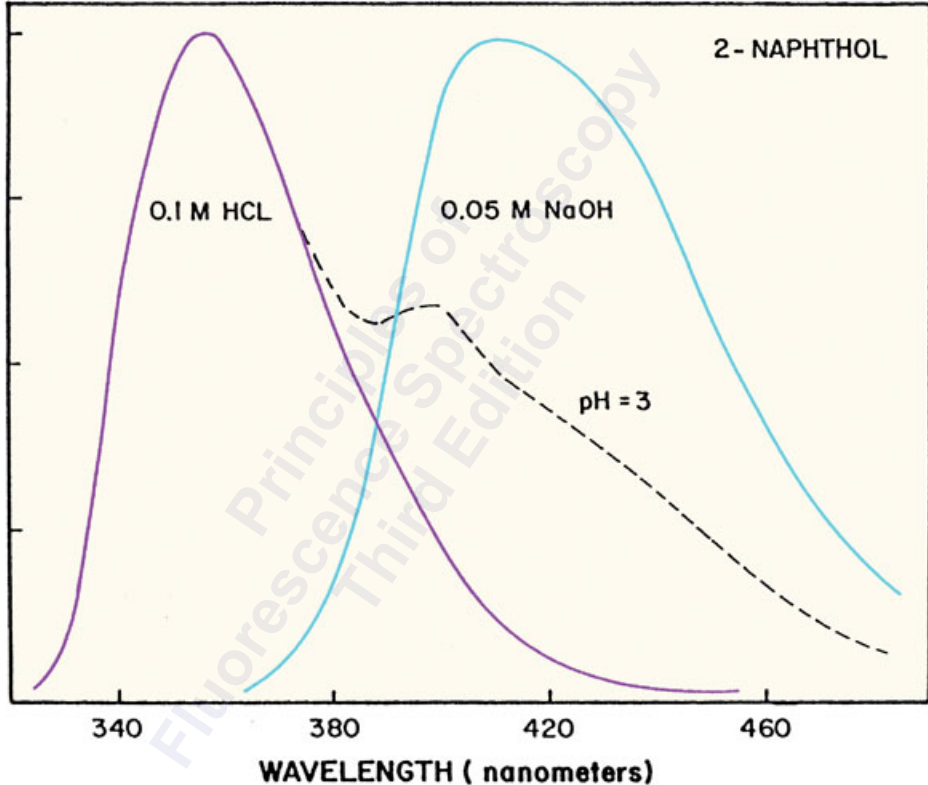
340

380

420

460

WAVELENGTH ( nanometers)



NORMALIZED ABSORBANCE

1.0  
0.5  
0

300

325

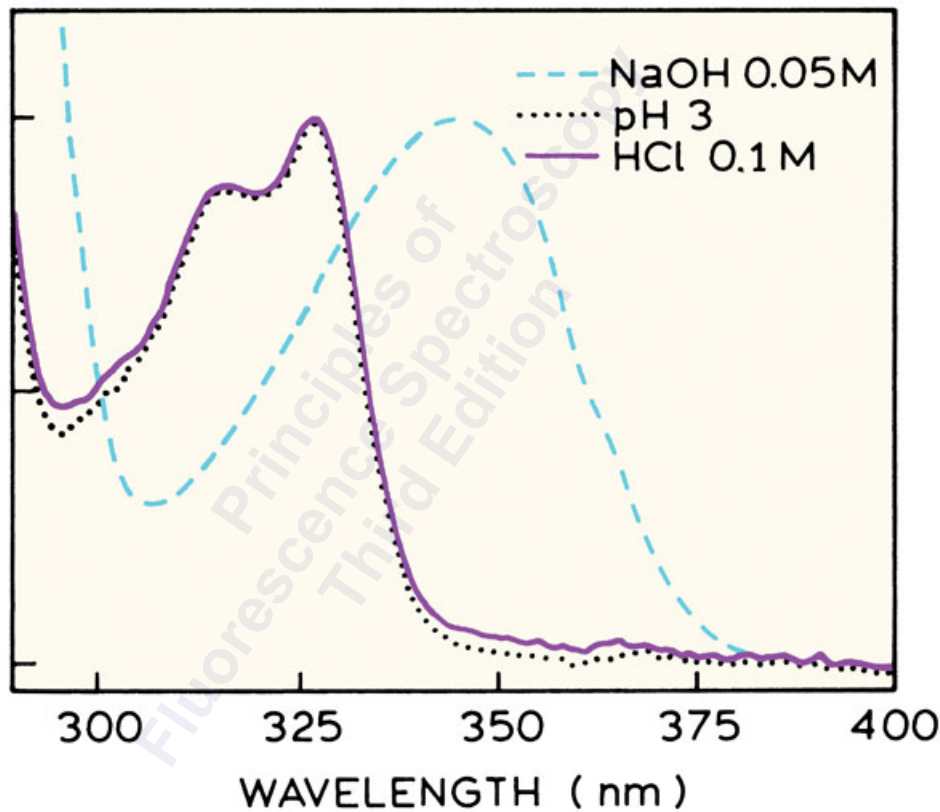
350

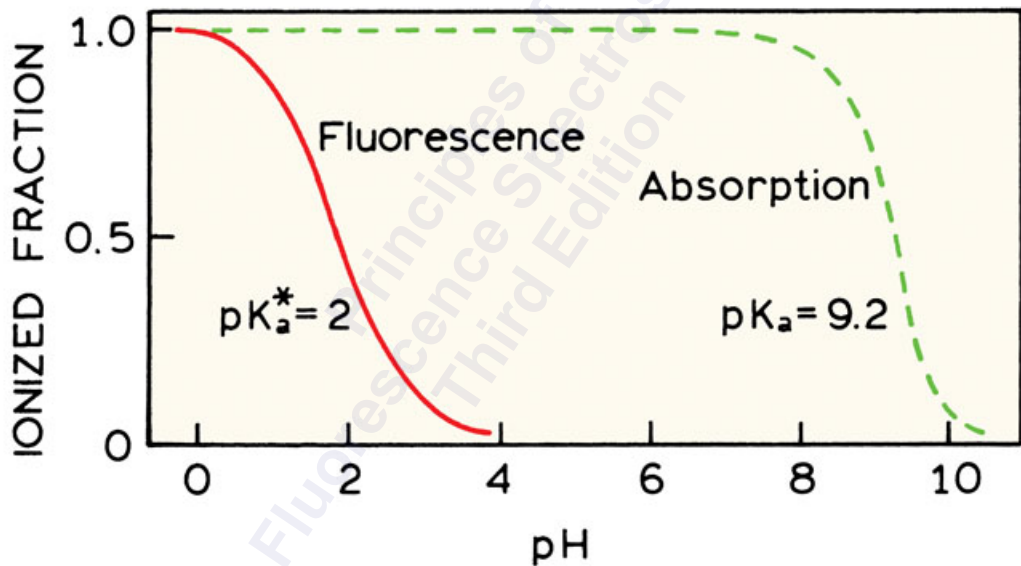
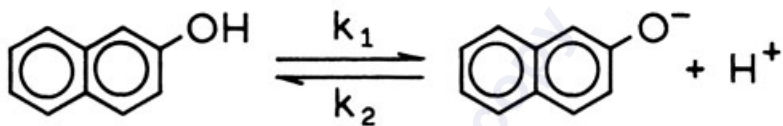
375

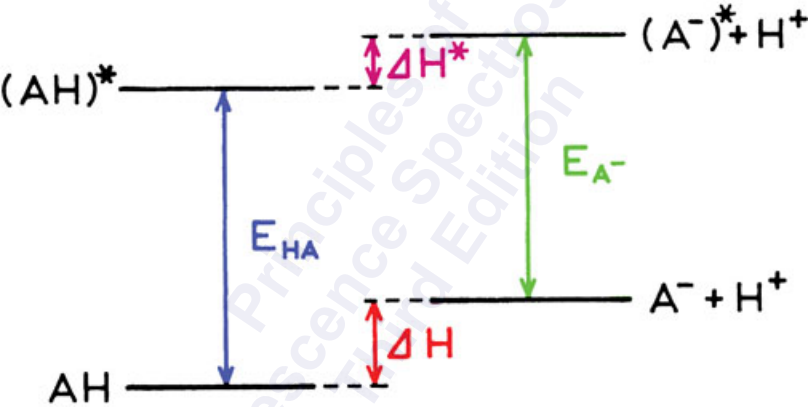
400

WAVELENGTH ( nm )

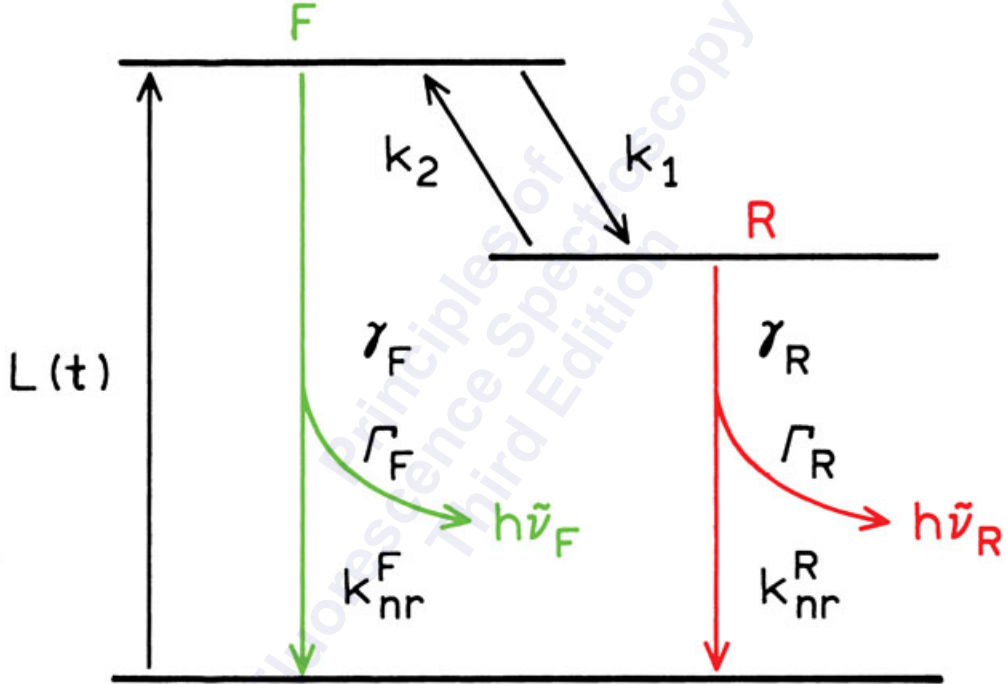
NaOH 0.05M  
pH 3  
HCl 0.1 M

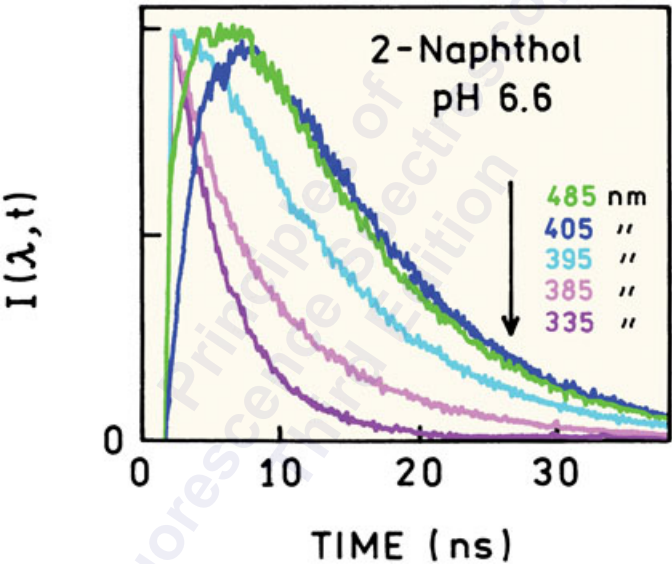




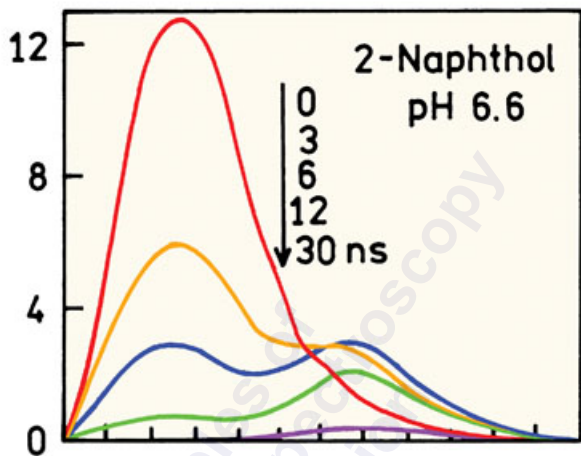




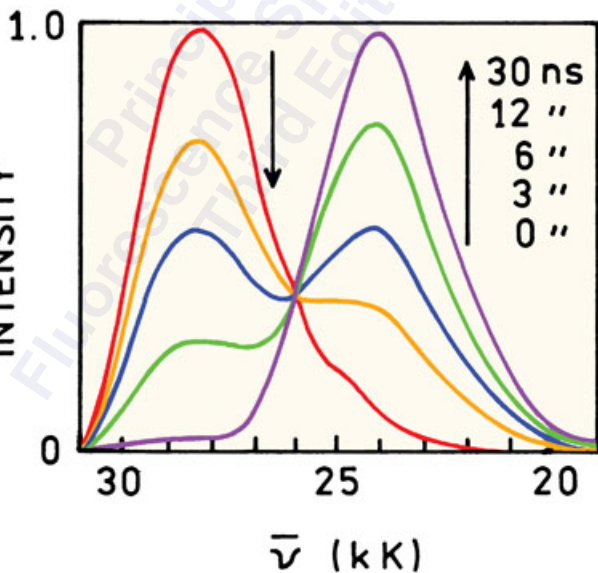


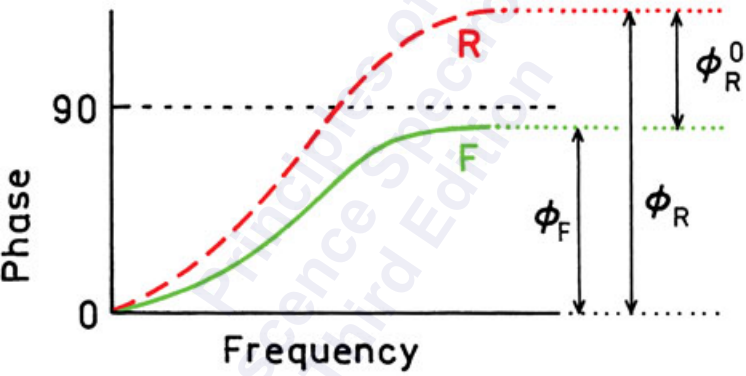


INTENSITY



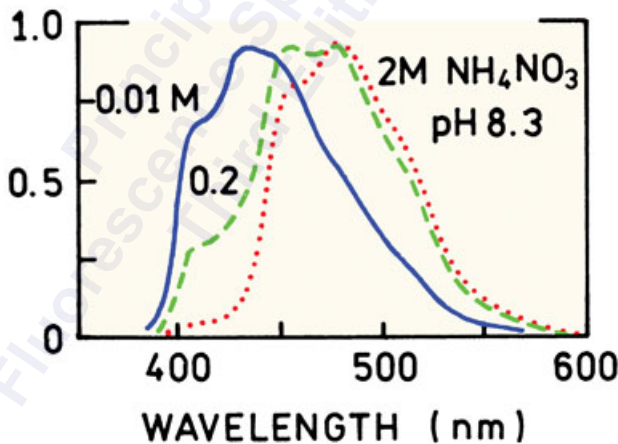
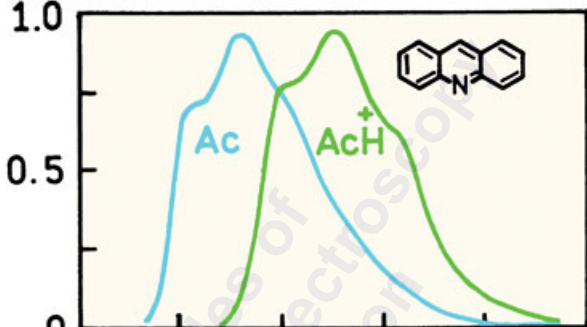
AREA NORMALIZED INTENSITY



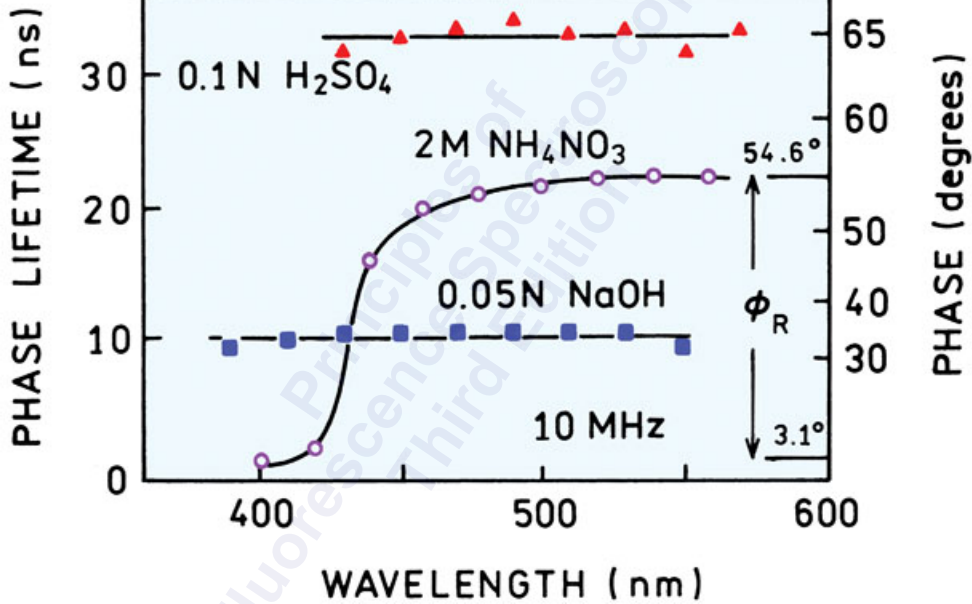


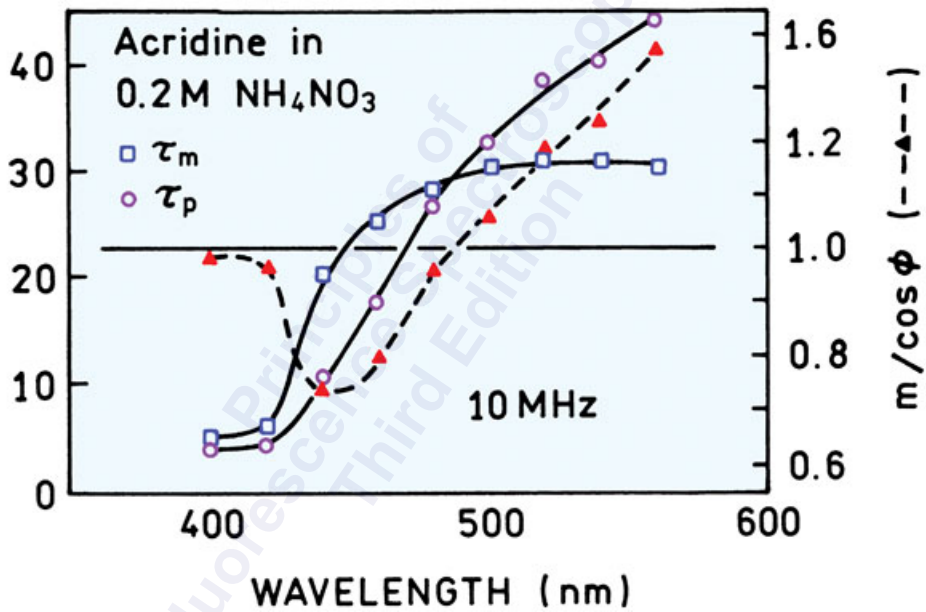
# STEADY STATE

FLUORESCENCE INTENSITY (normalized)



APPARENT  
PHASE LIFETIME (ns)





FLUORESCENCE INTENSITY

2PI in p-Xylene  
20°C

$10^{-5}$  M

$6 \times 10^{-3}$  M

F ↓

↓

↓

↓

↓

↓

R ↓

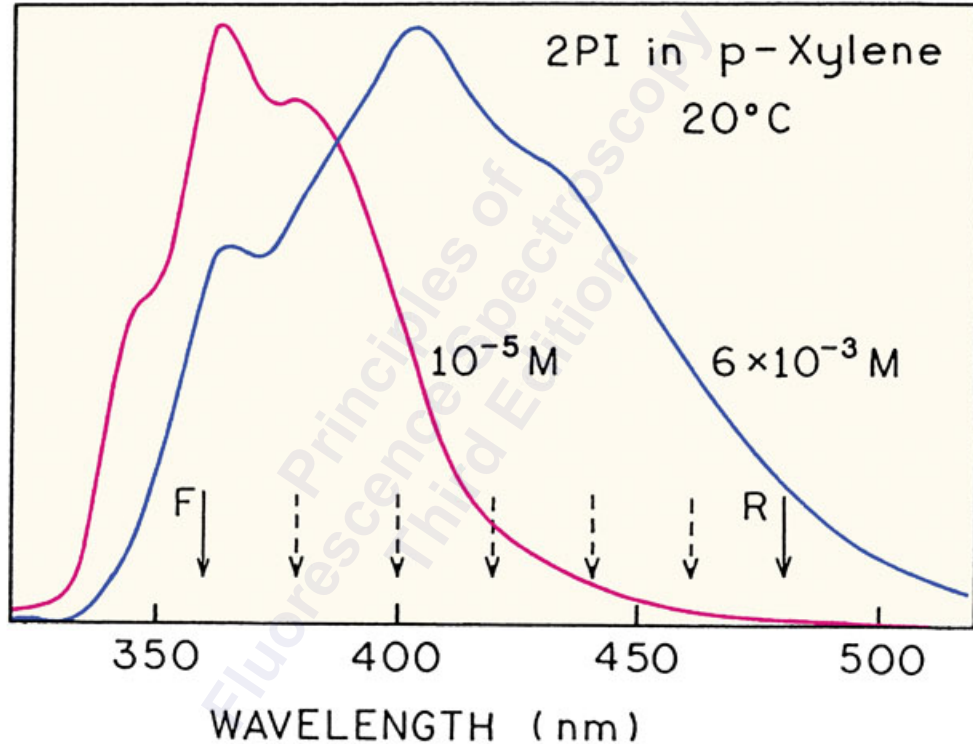
350

400

450

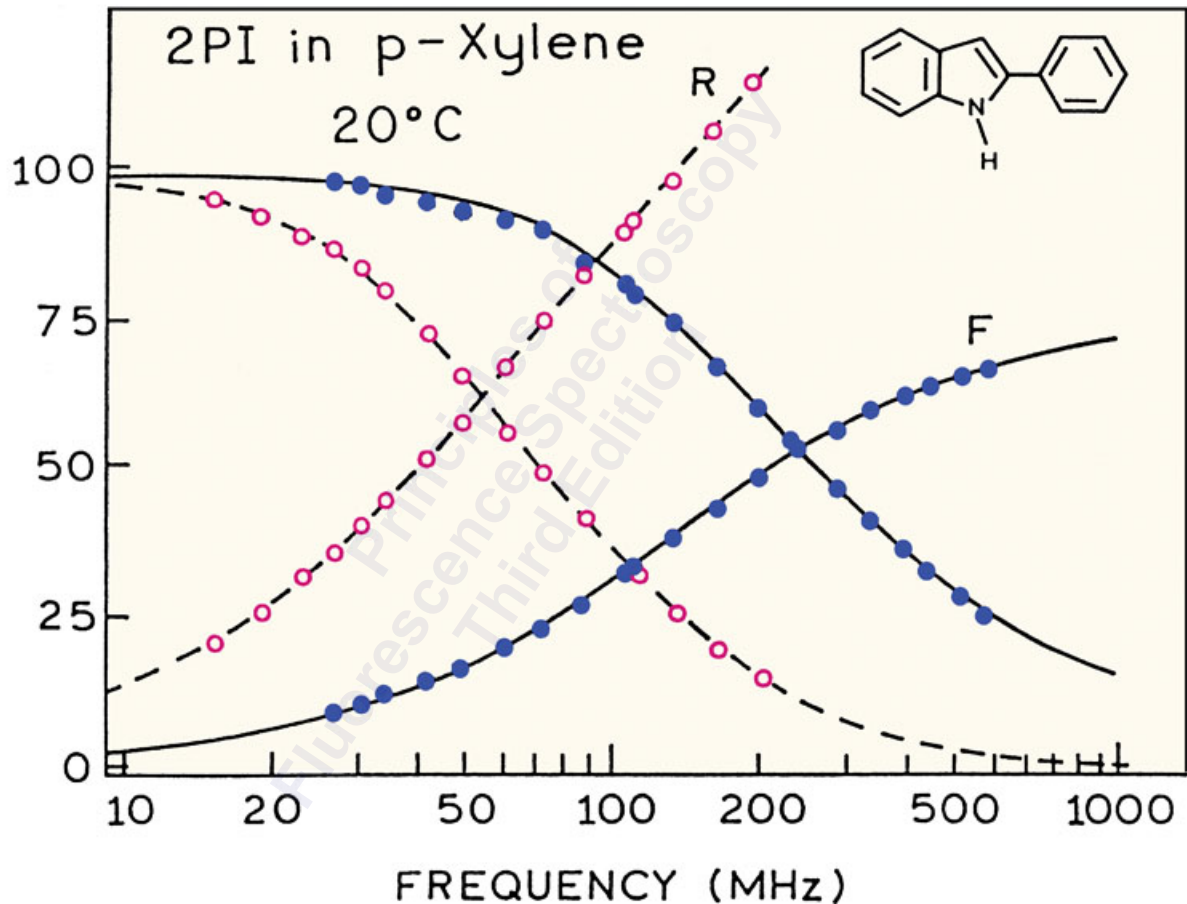
500

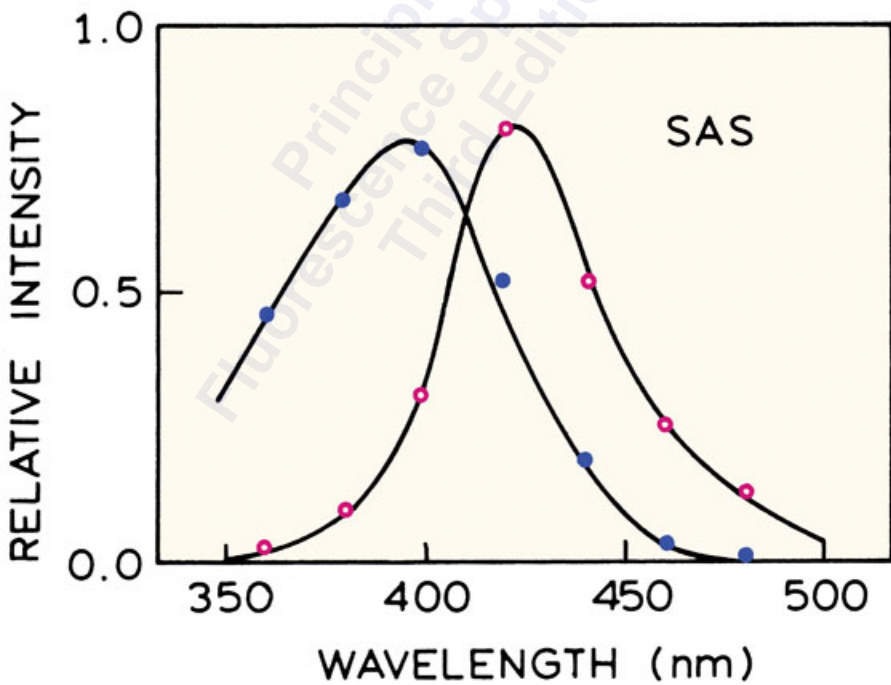
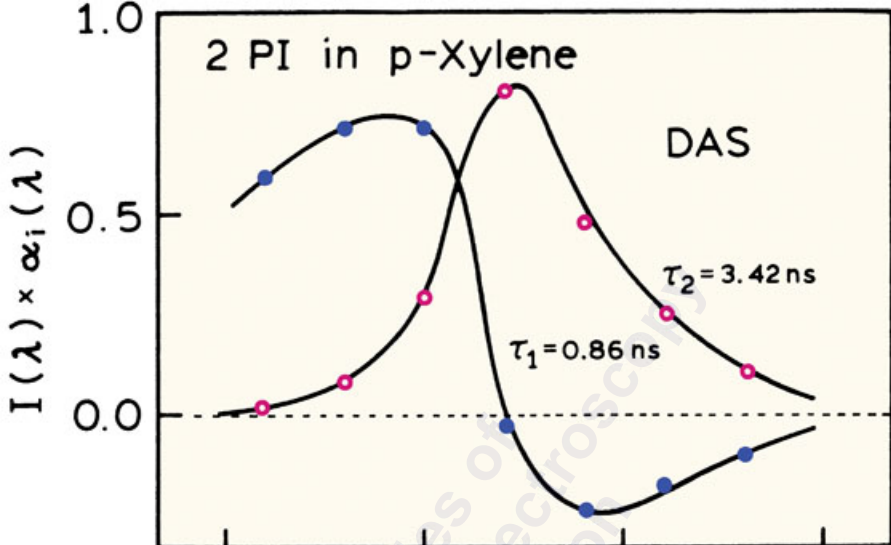
WAVELENGTH (nm)

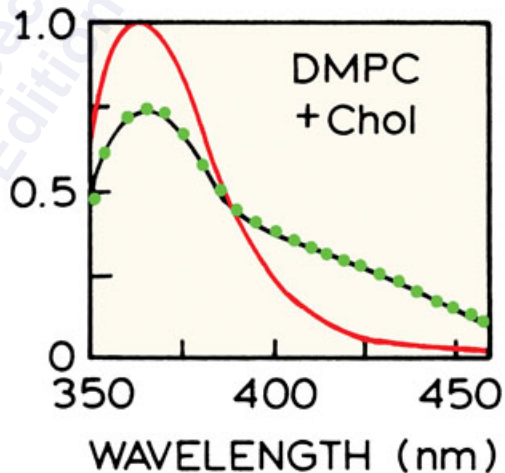
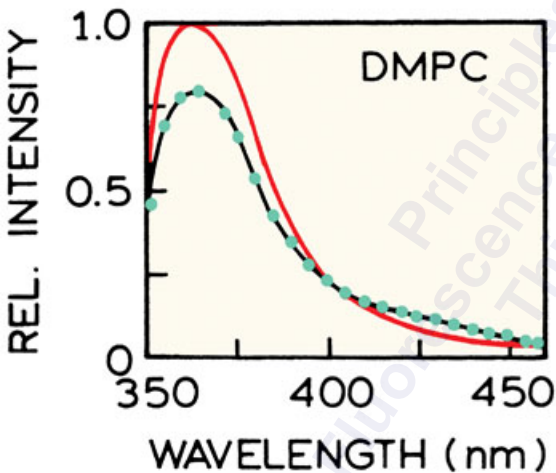
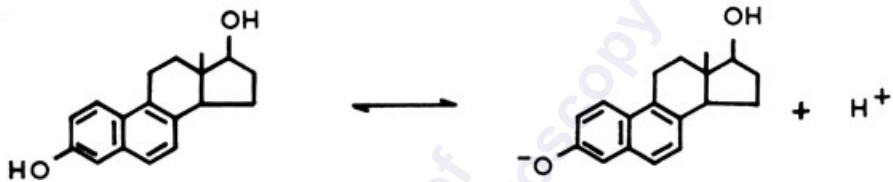




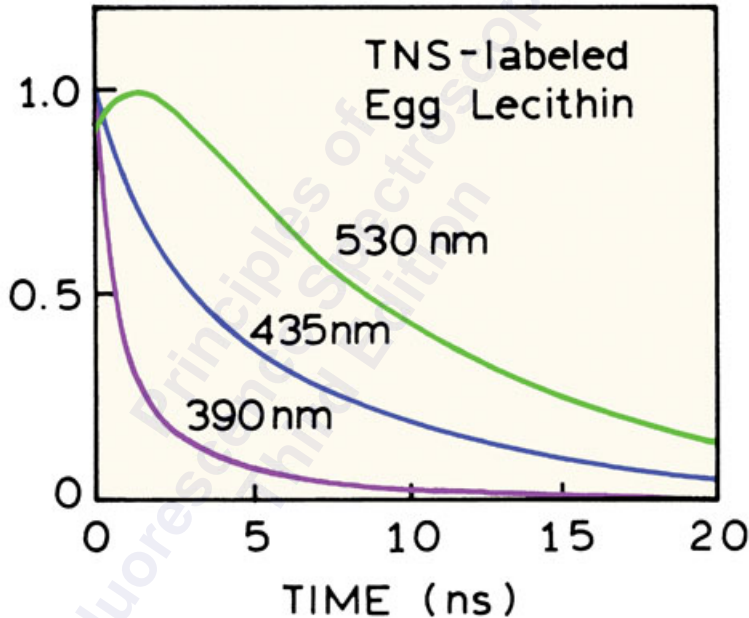
PHASE ANGLE (degrees)  
or MODULATION (%)



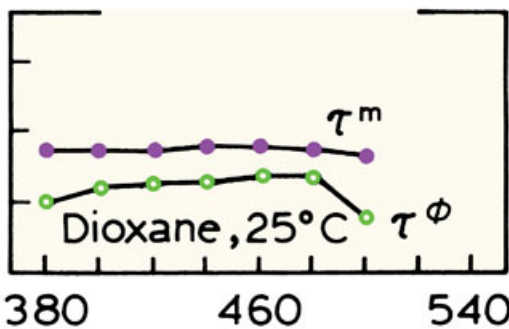
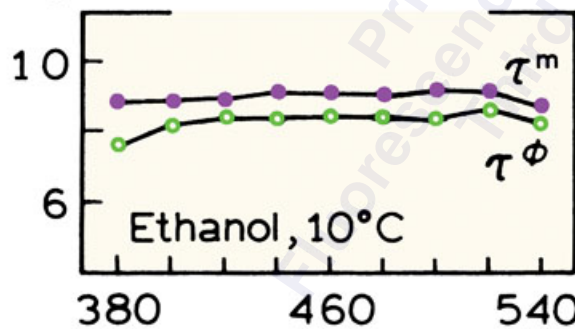
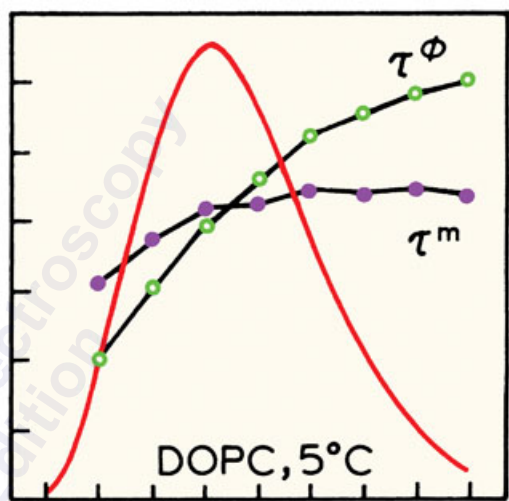
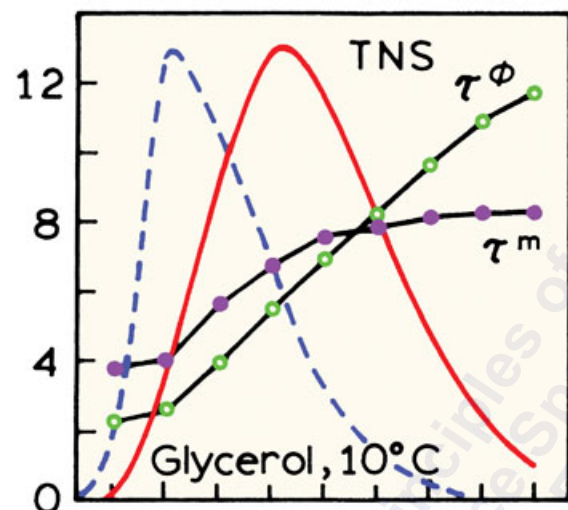




NORMALIZED  
IMPULSE RESPONSE

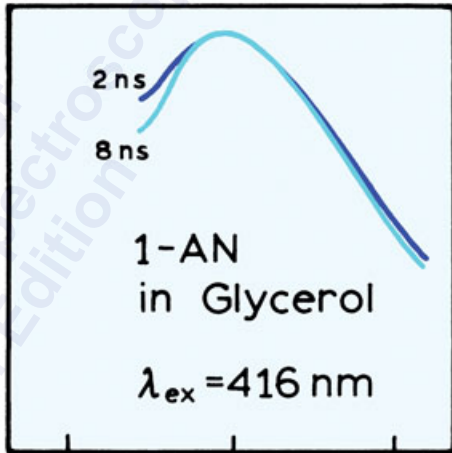
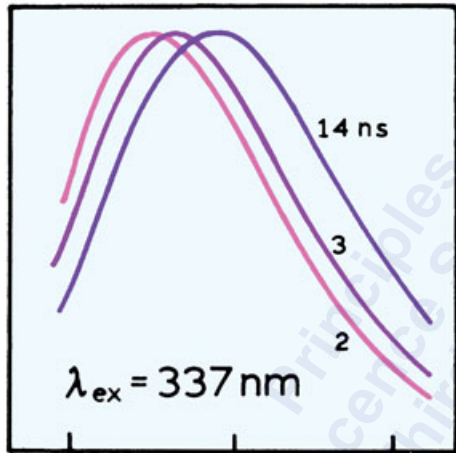


FLUORESCENCE LIFETIME (ns)



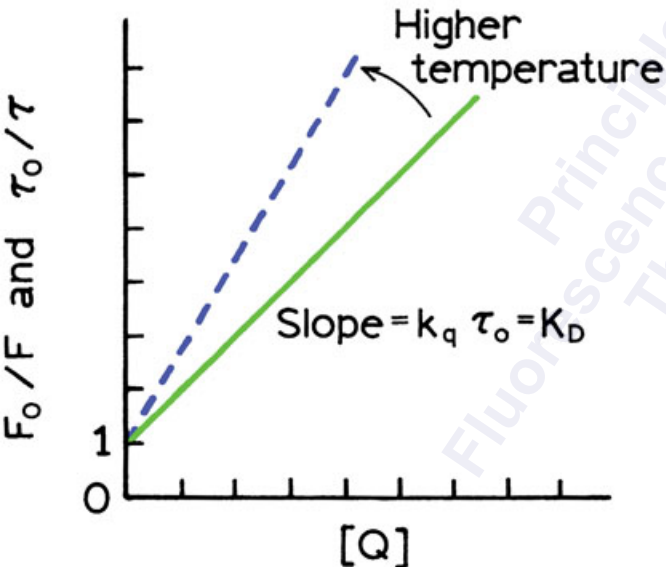
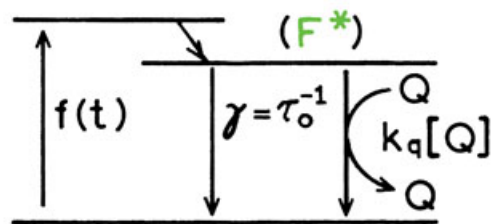
WAVELENGTH (nm)

INTENSITY

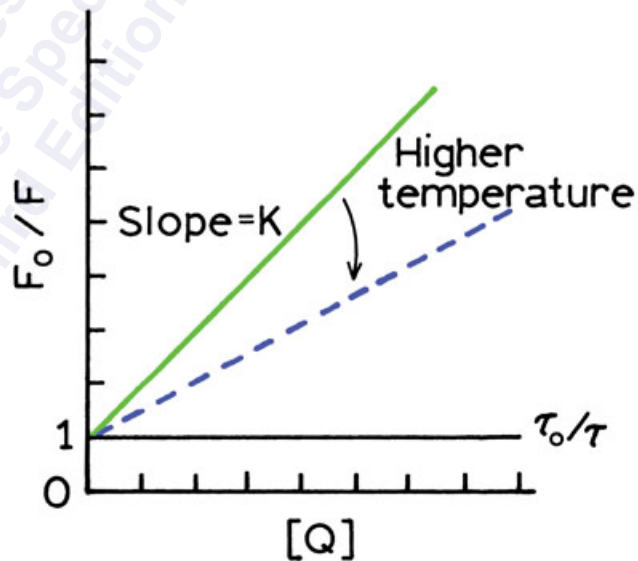
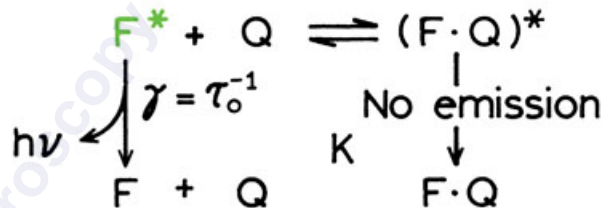


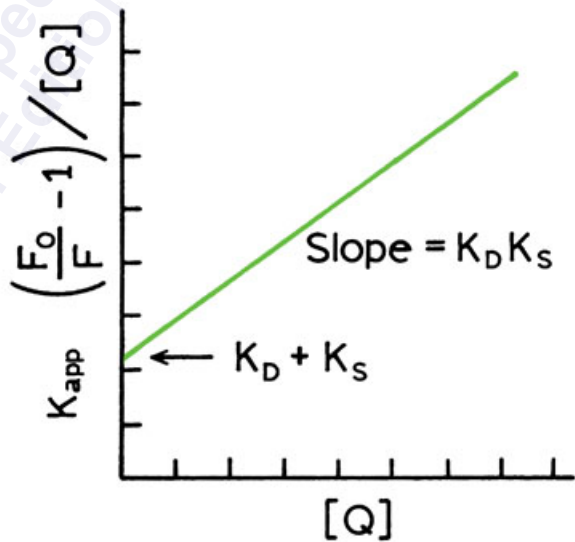
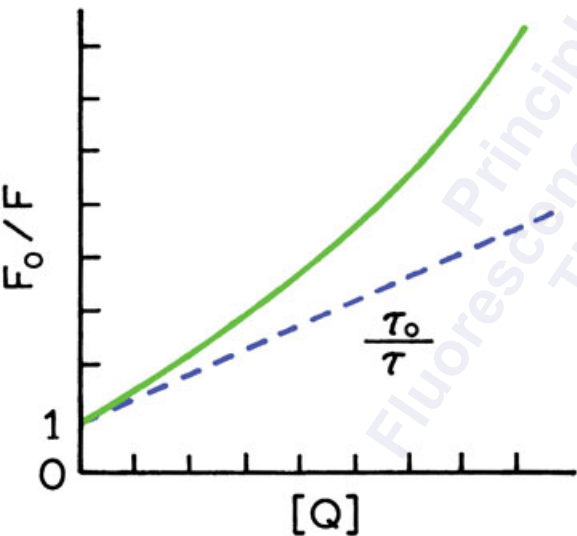
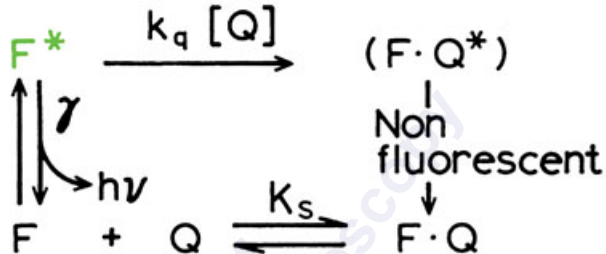
WAVELENGTH (nm)

## Collisional Quenching

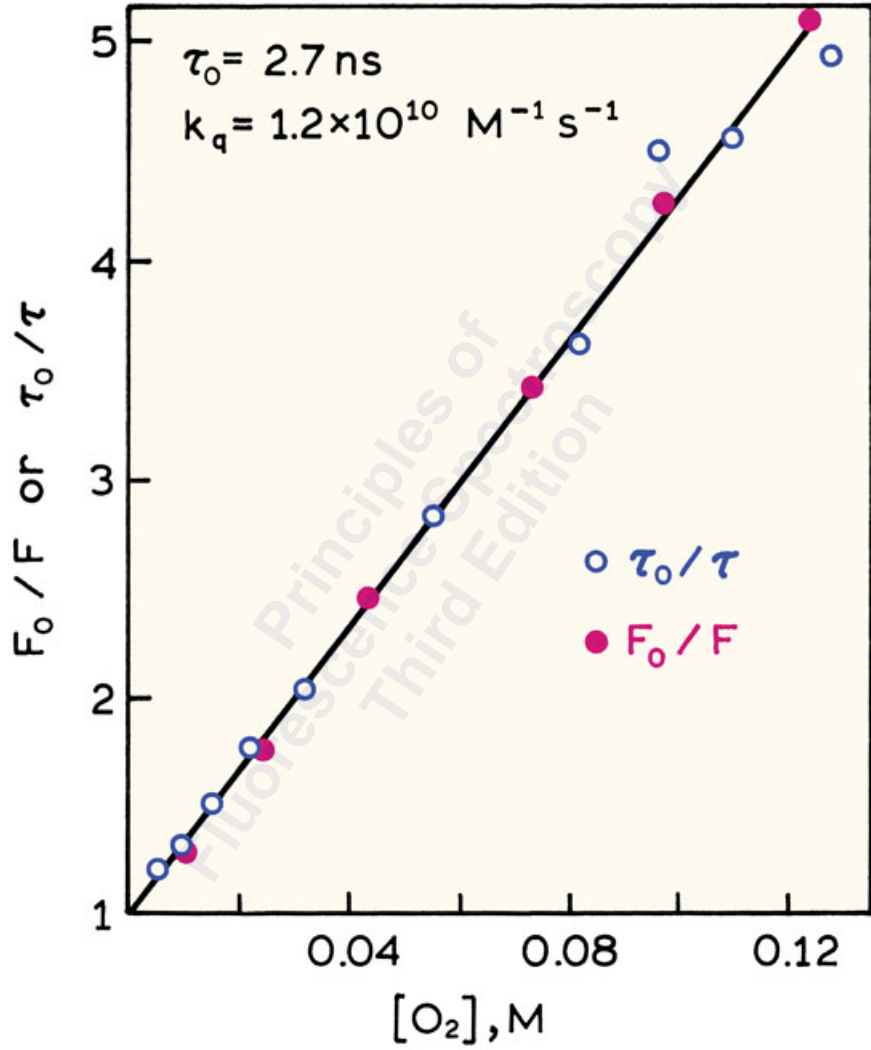


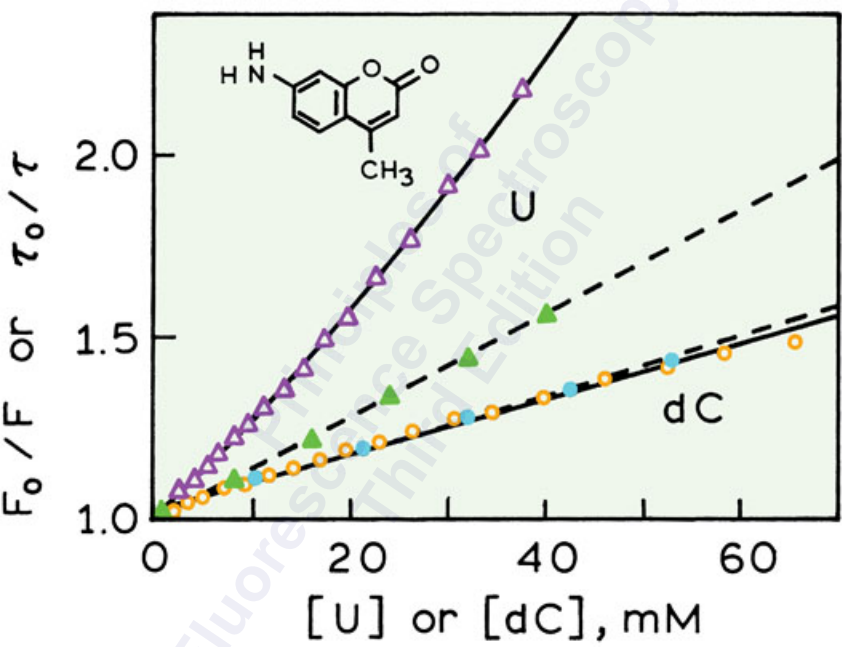
## Static Quenching

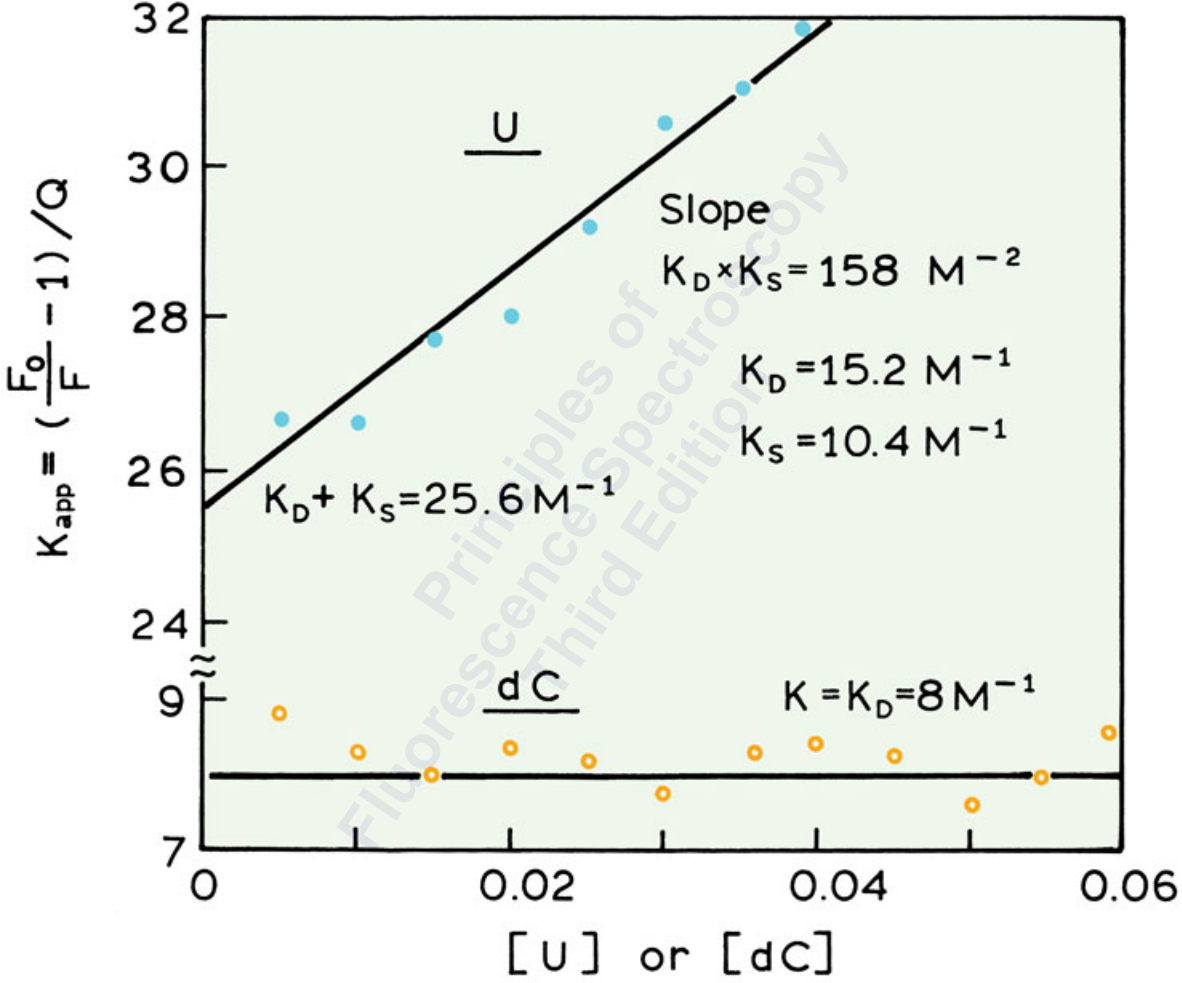




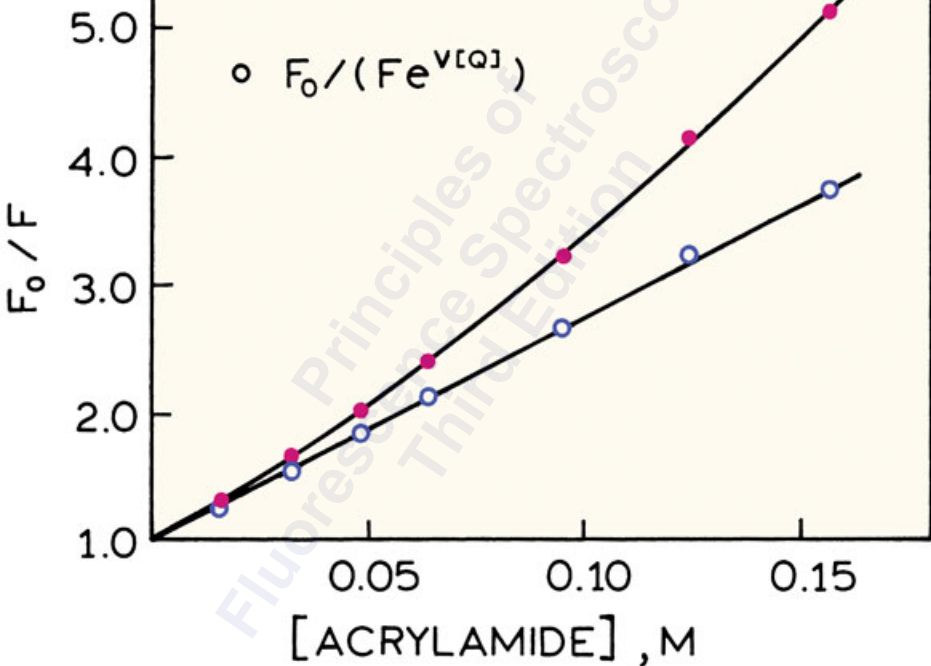


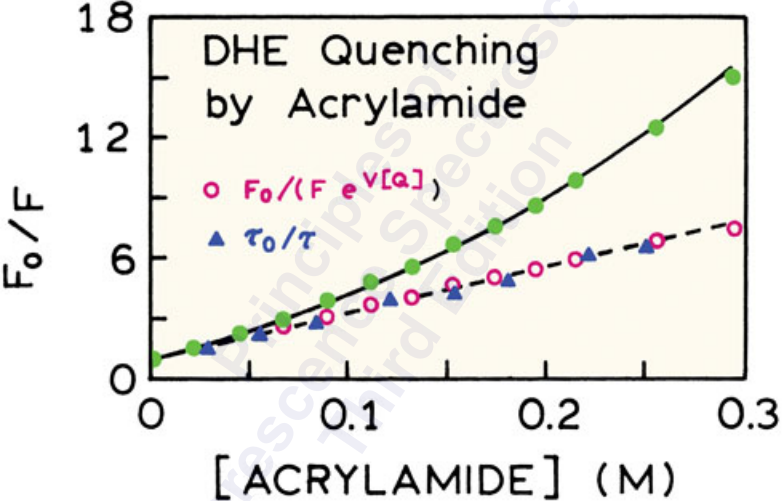


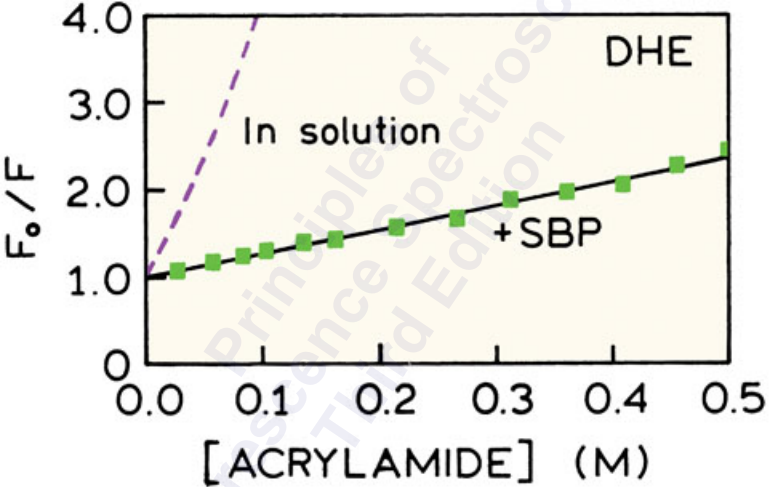


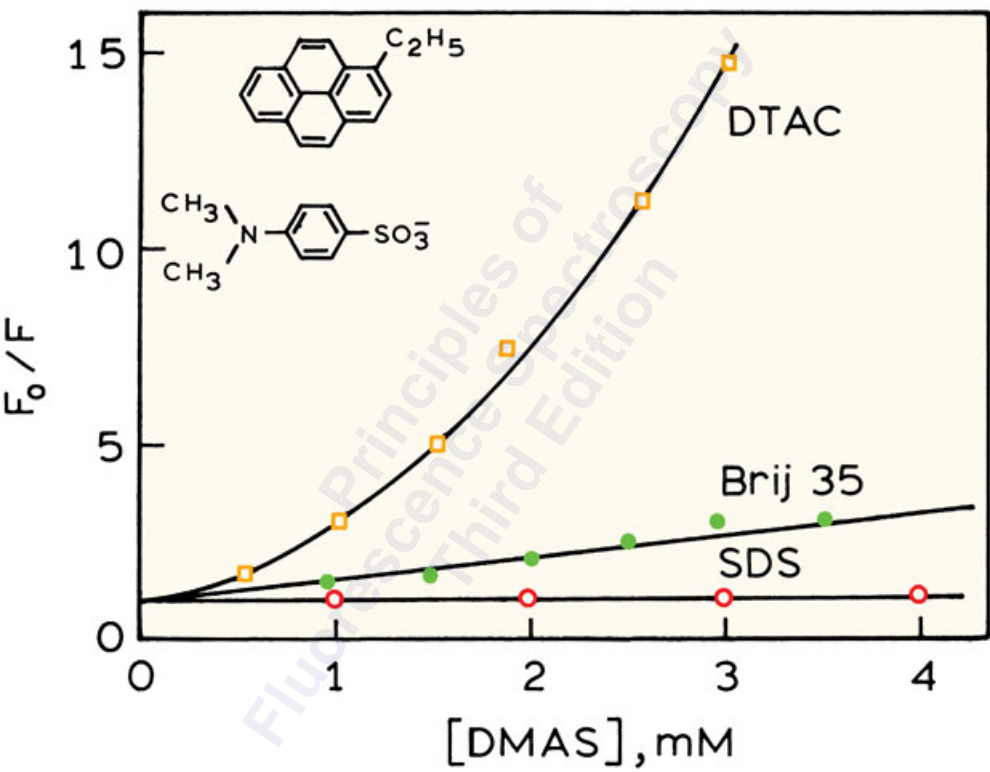


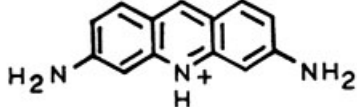
# NATA Quenching by Acrylamide







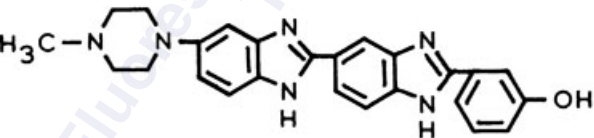




Proflavine

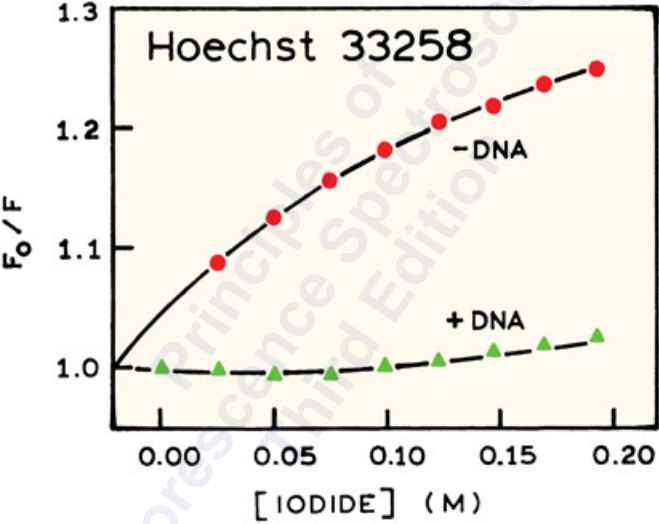


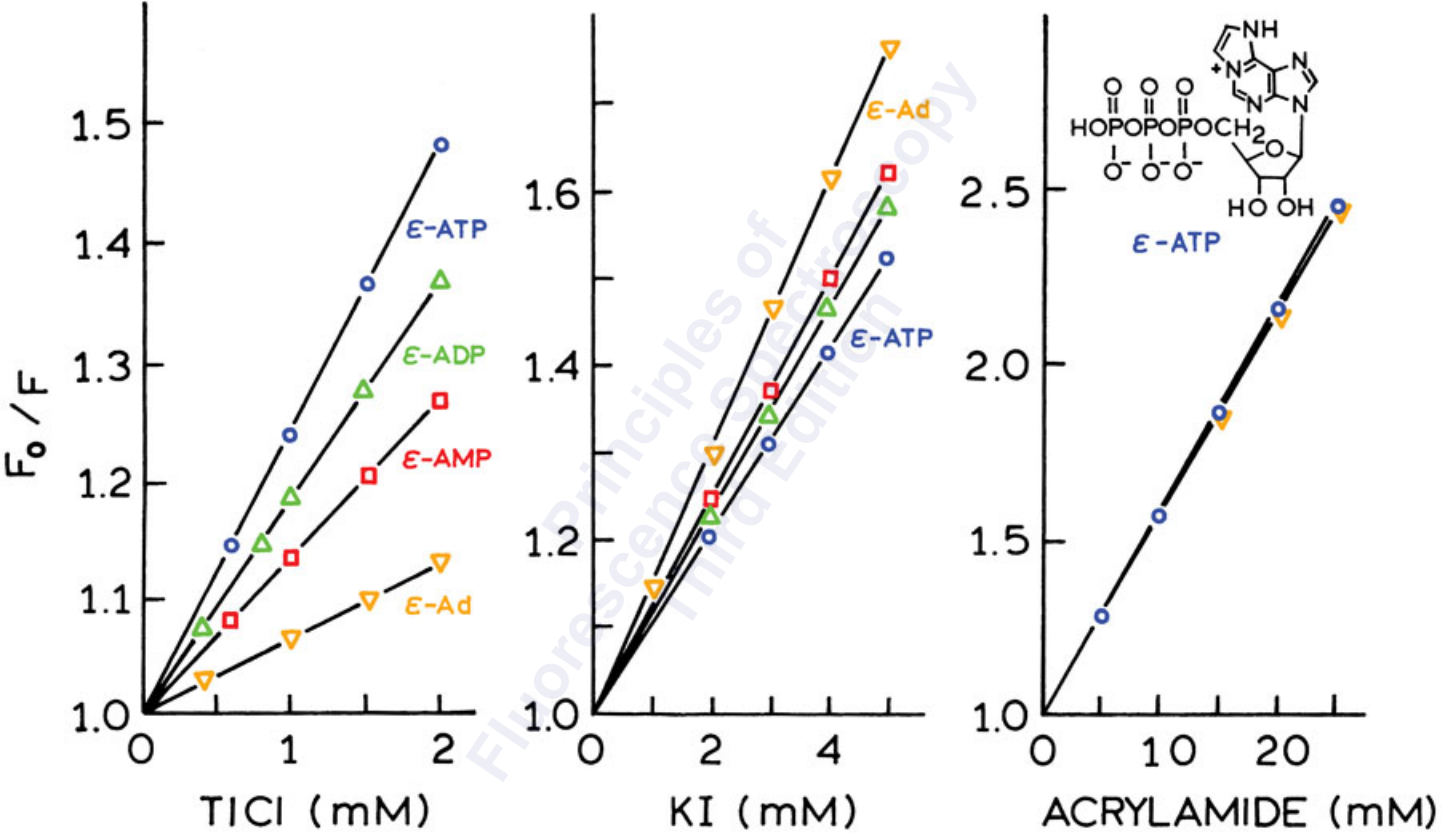
Coronene

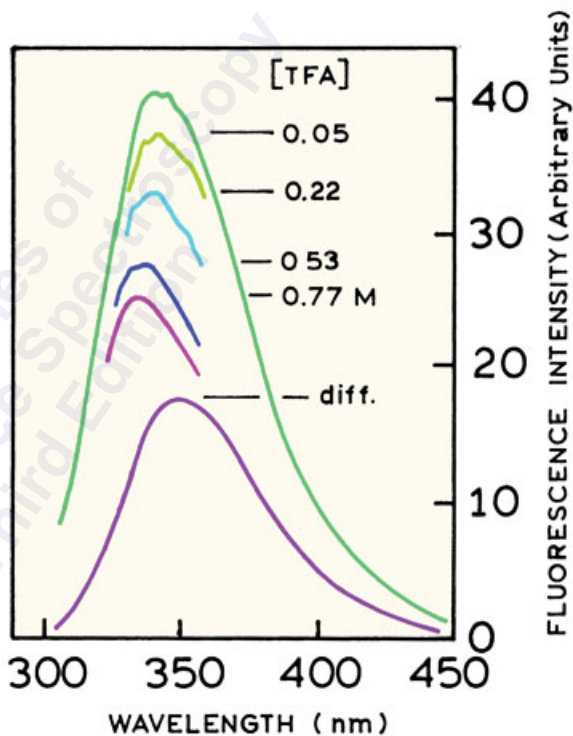
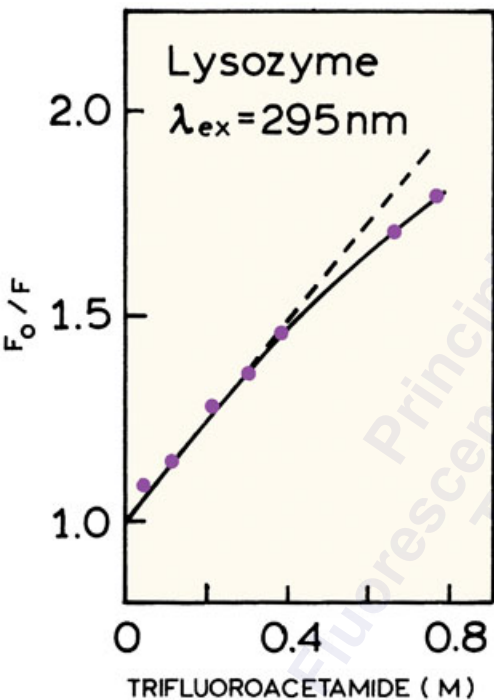


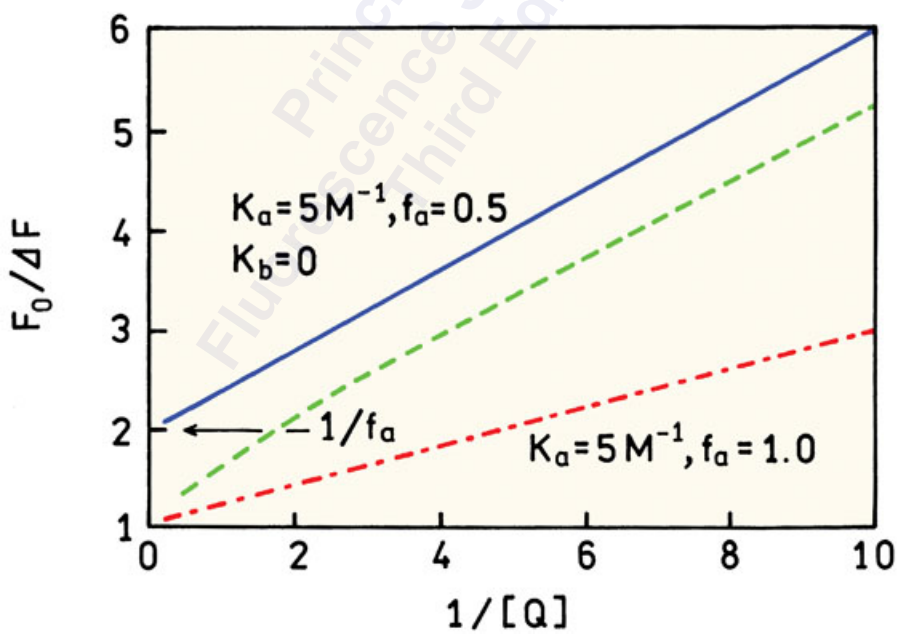
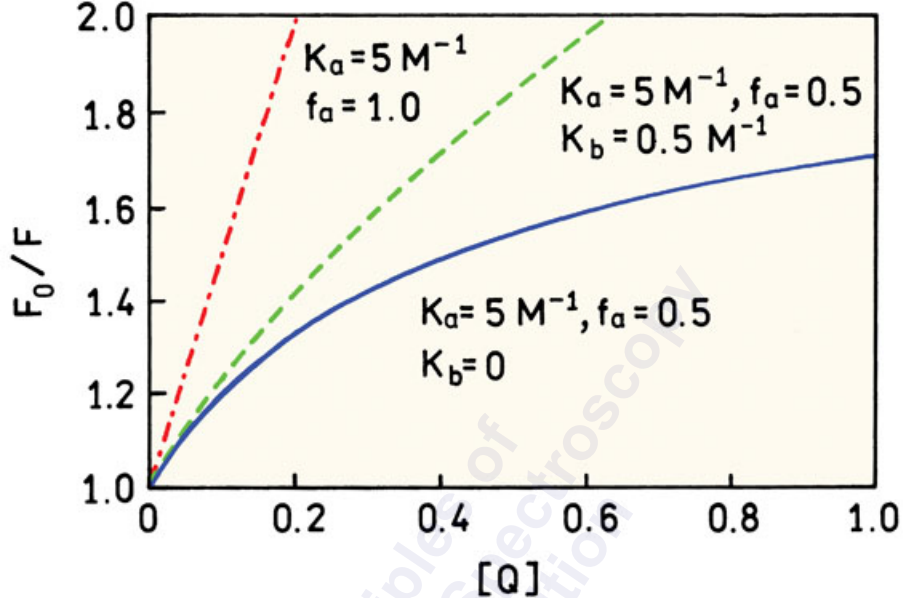
Hoechst 33258

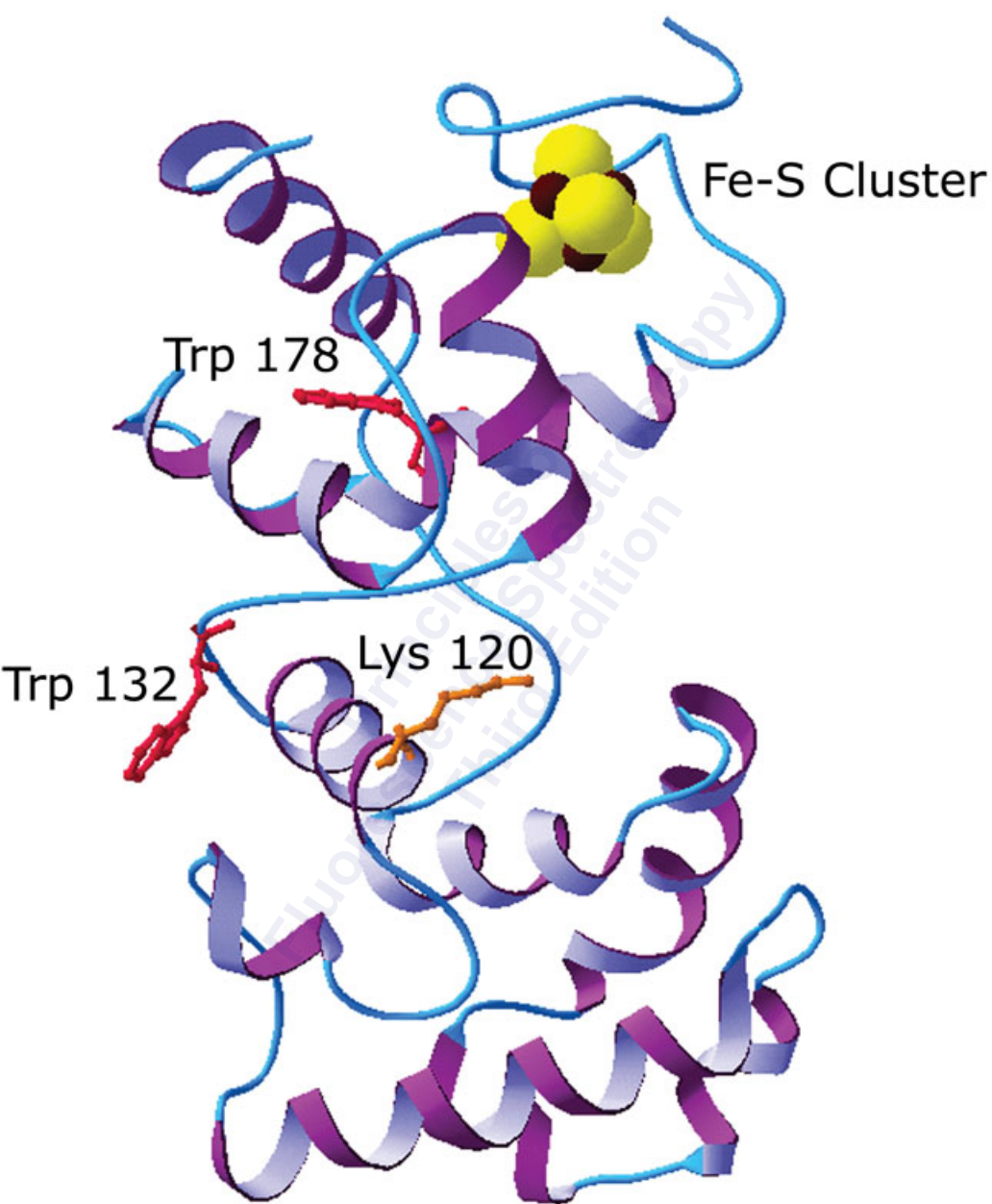


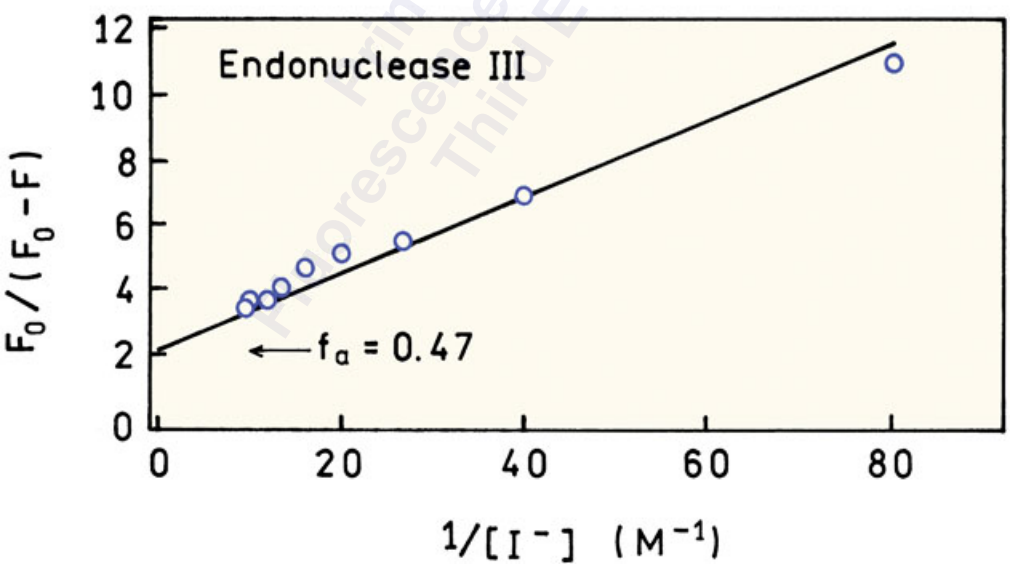
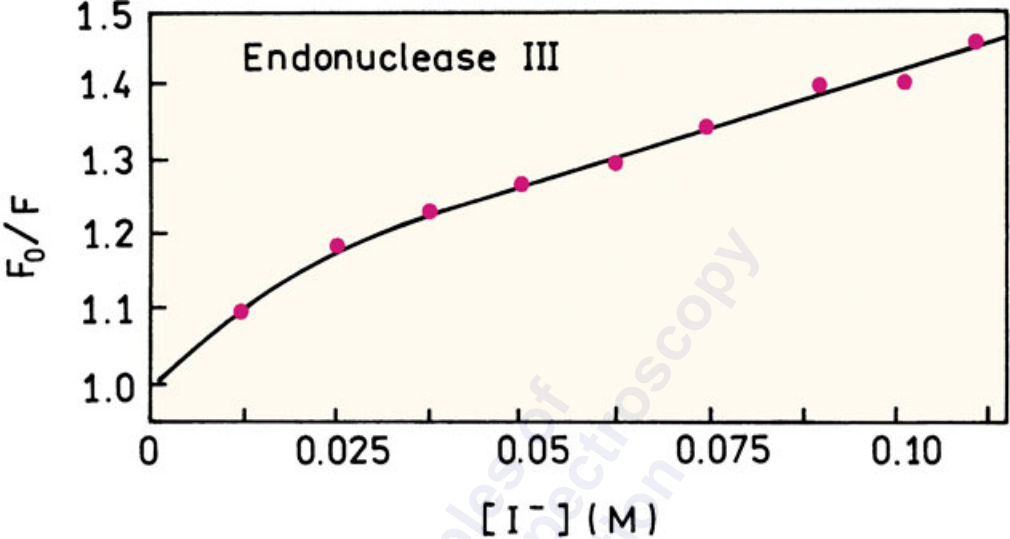


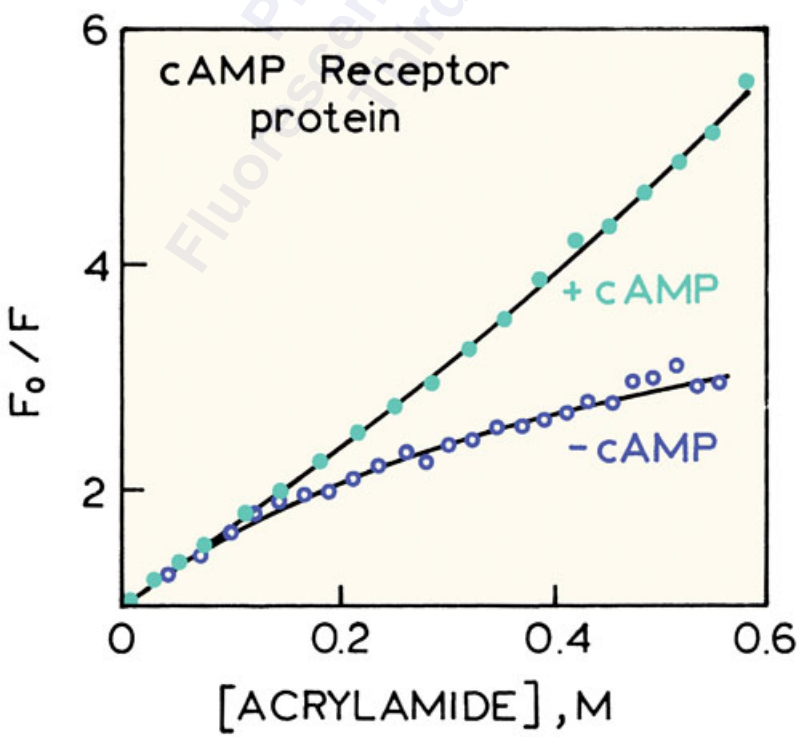
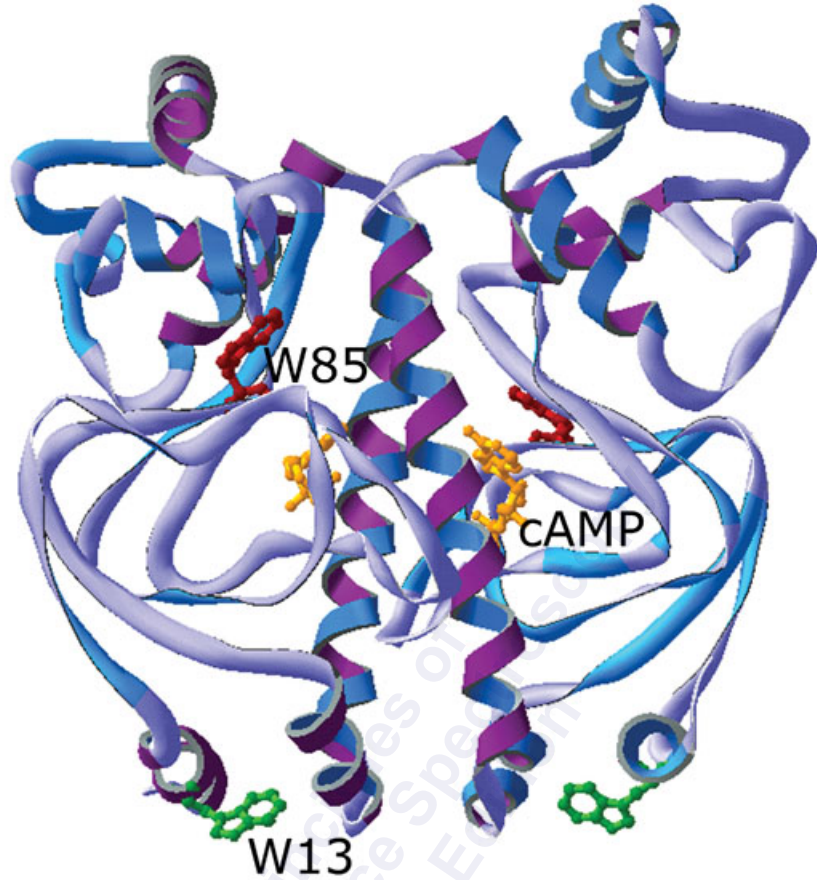


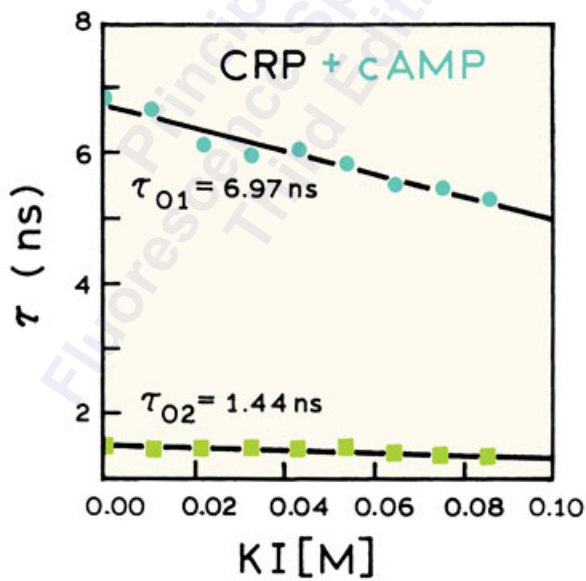
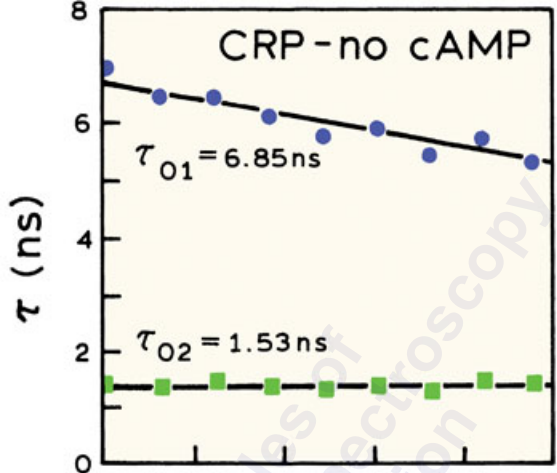




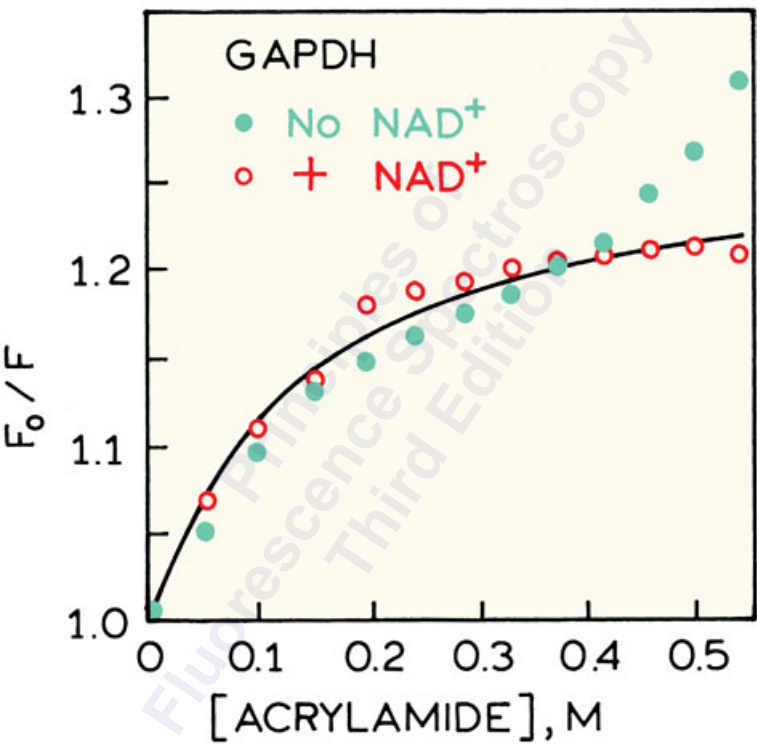


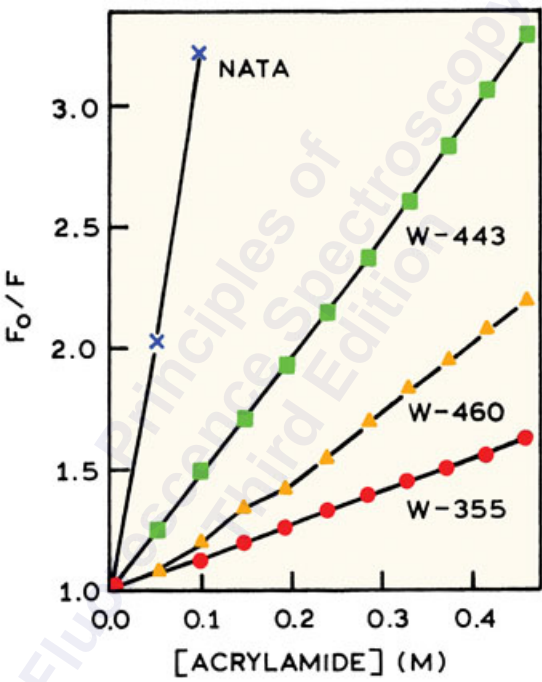


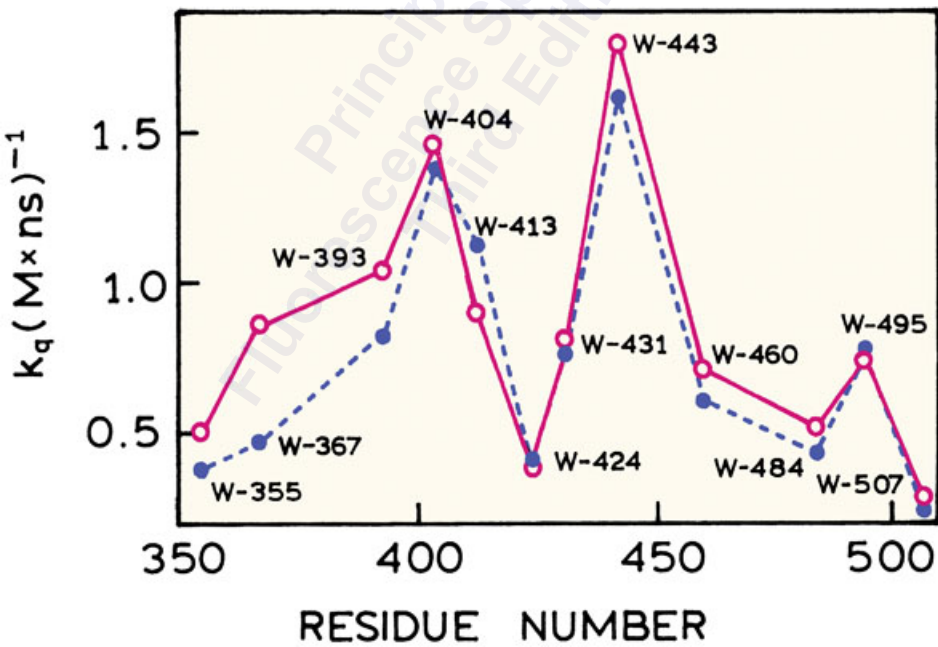
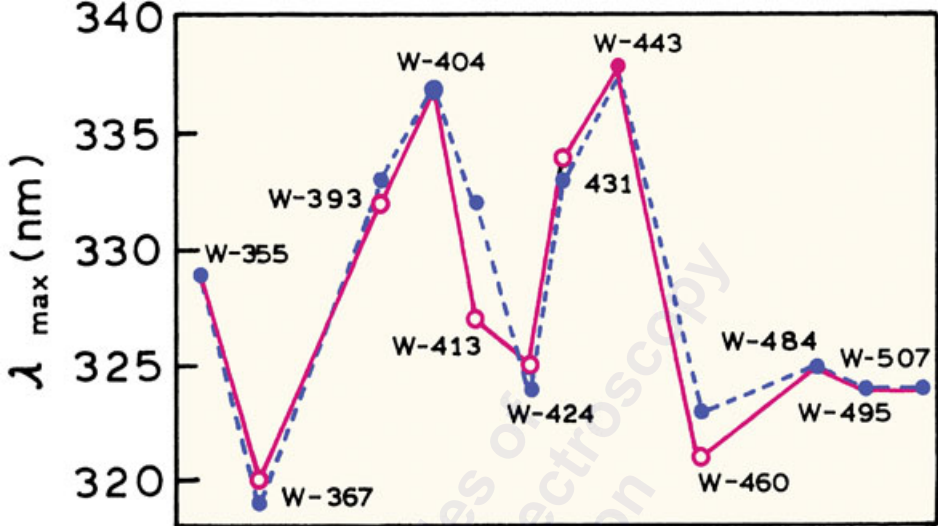


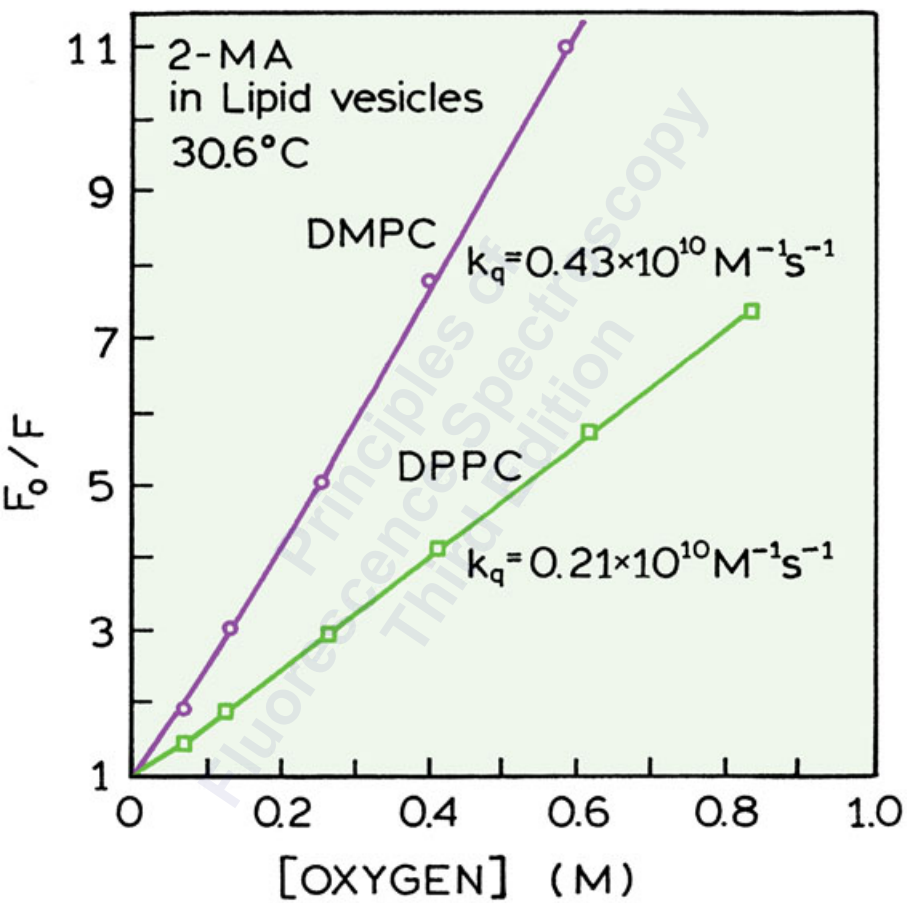


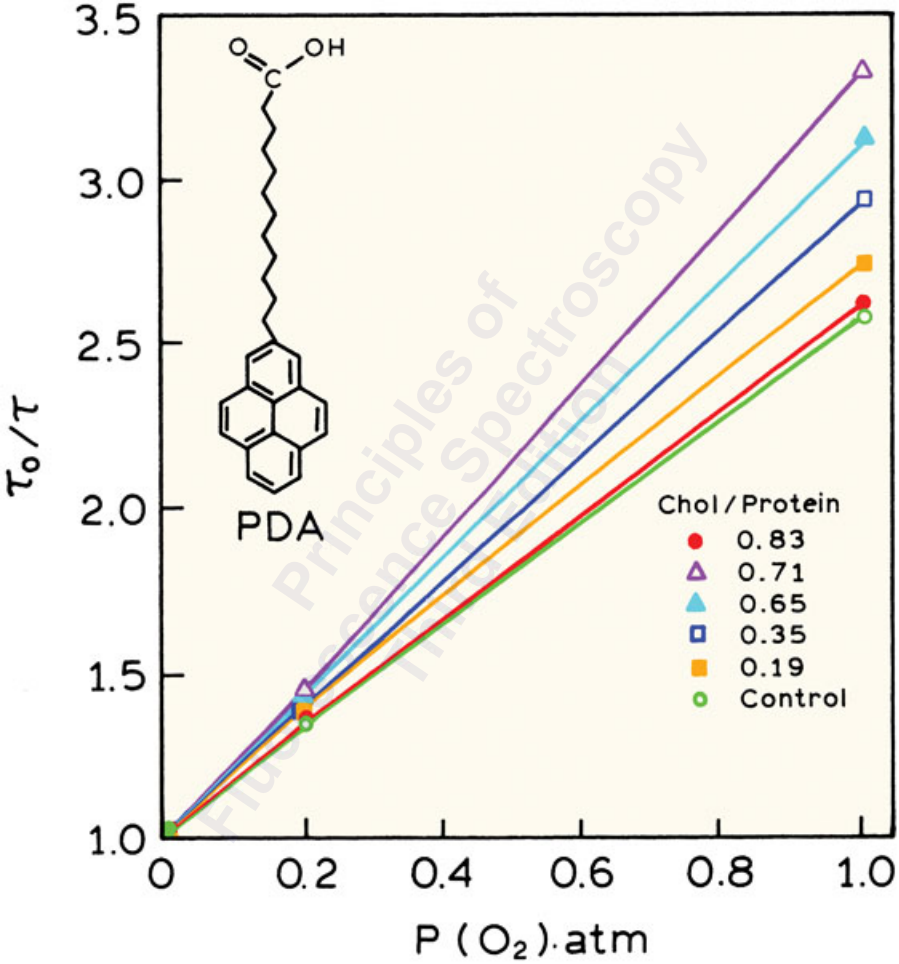


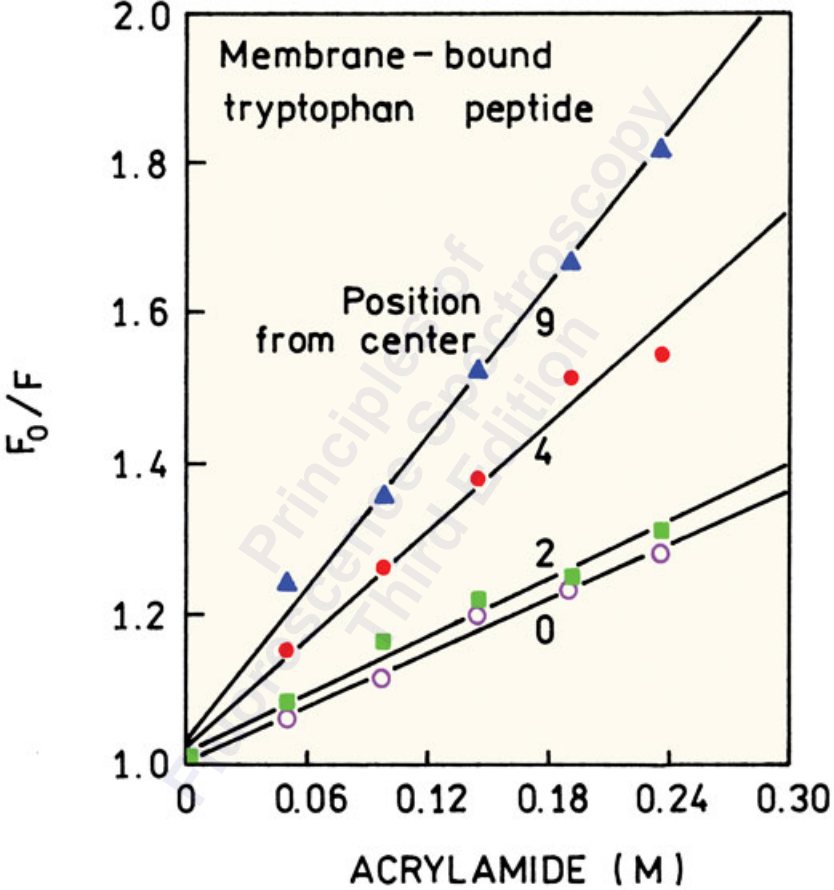


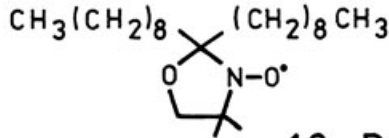




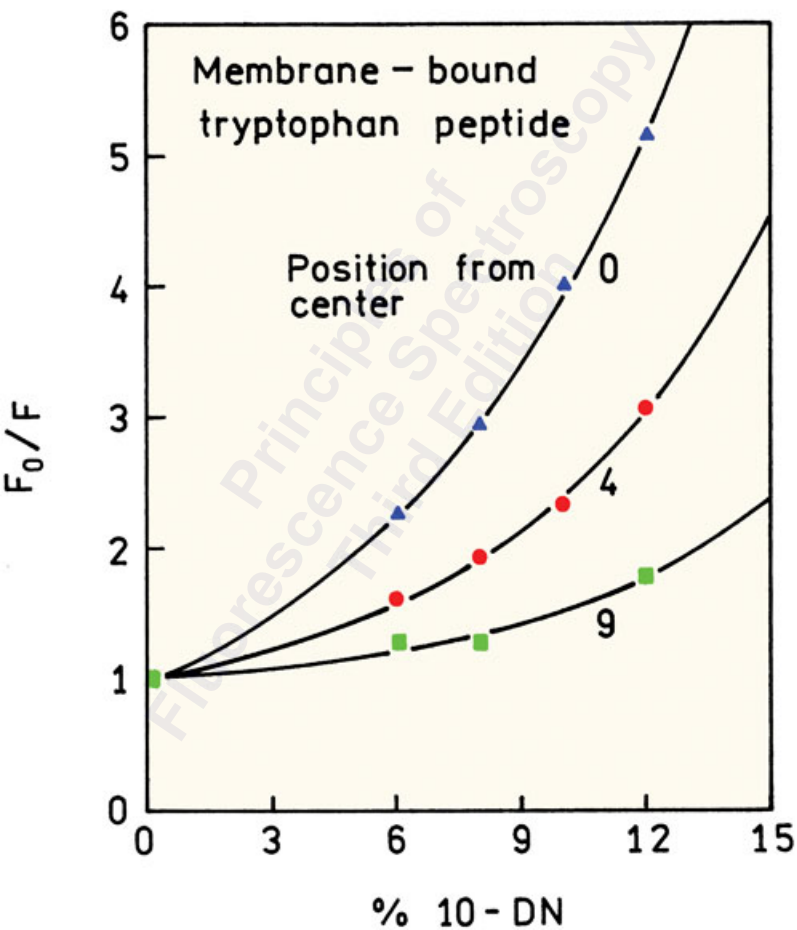


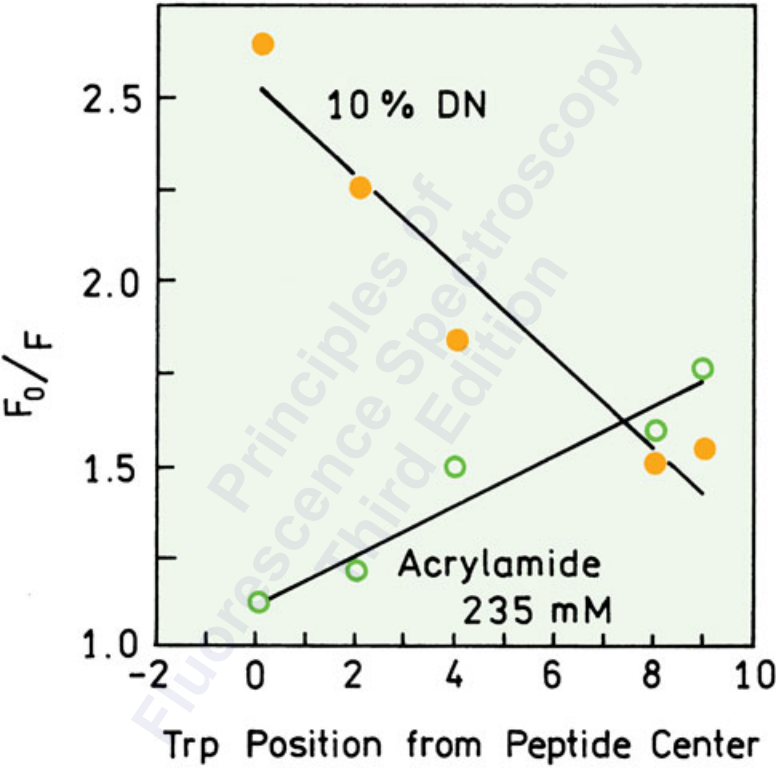






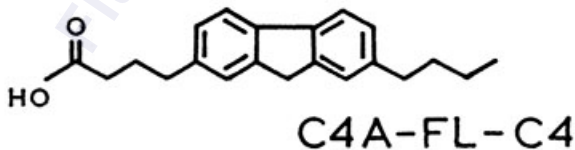
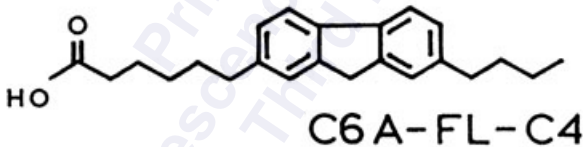
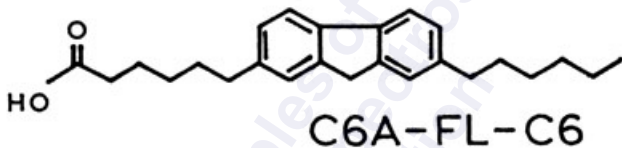
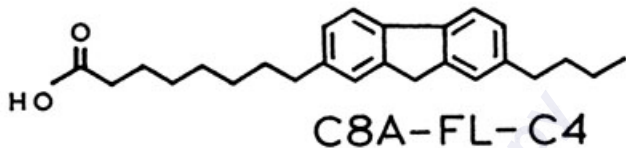
10-DN

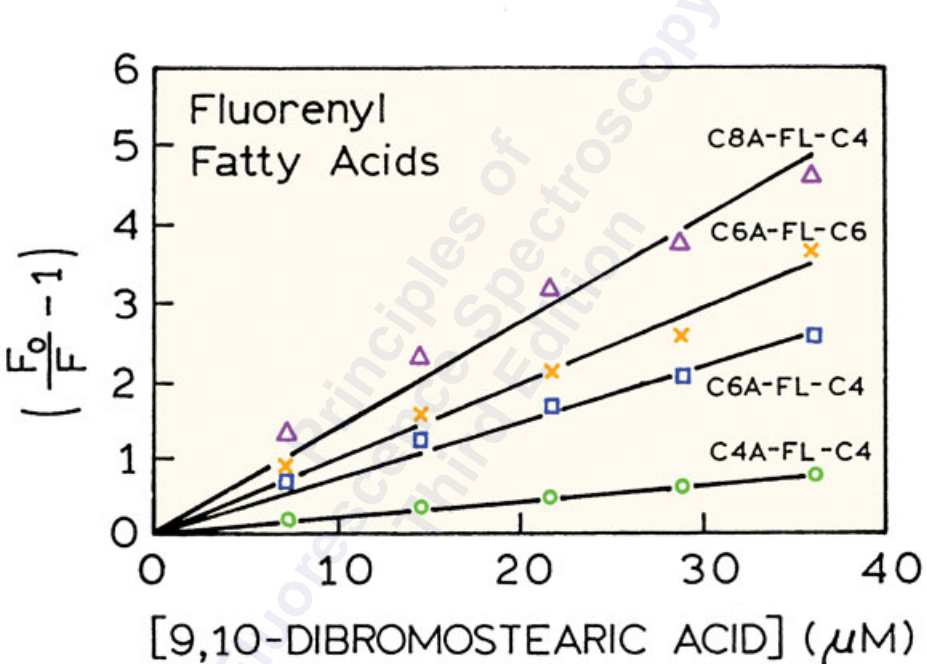


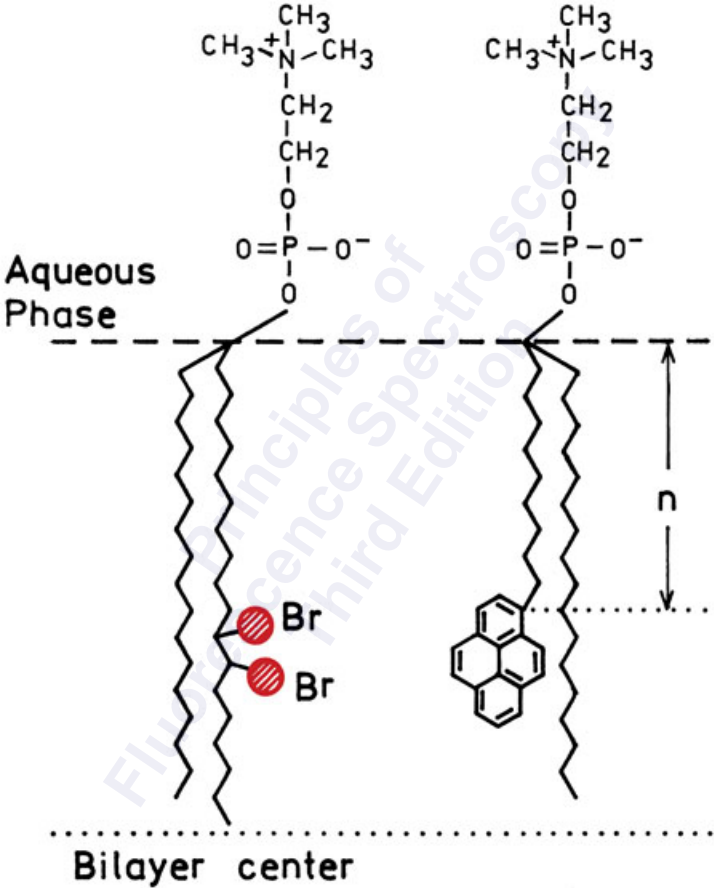


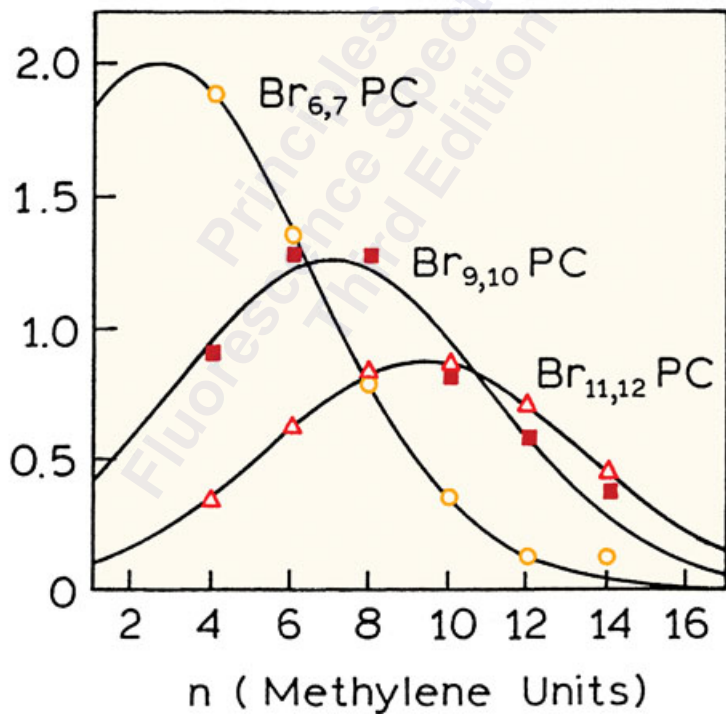
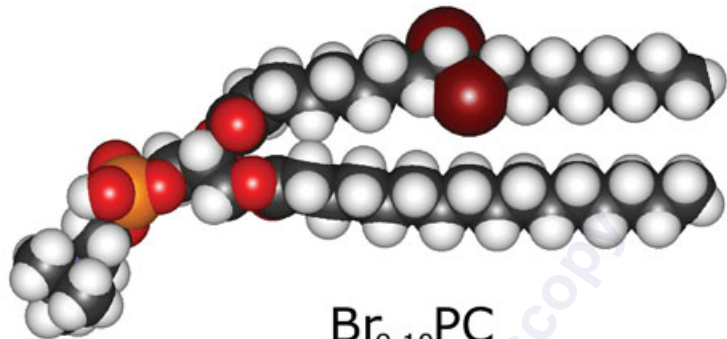


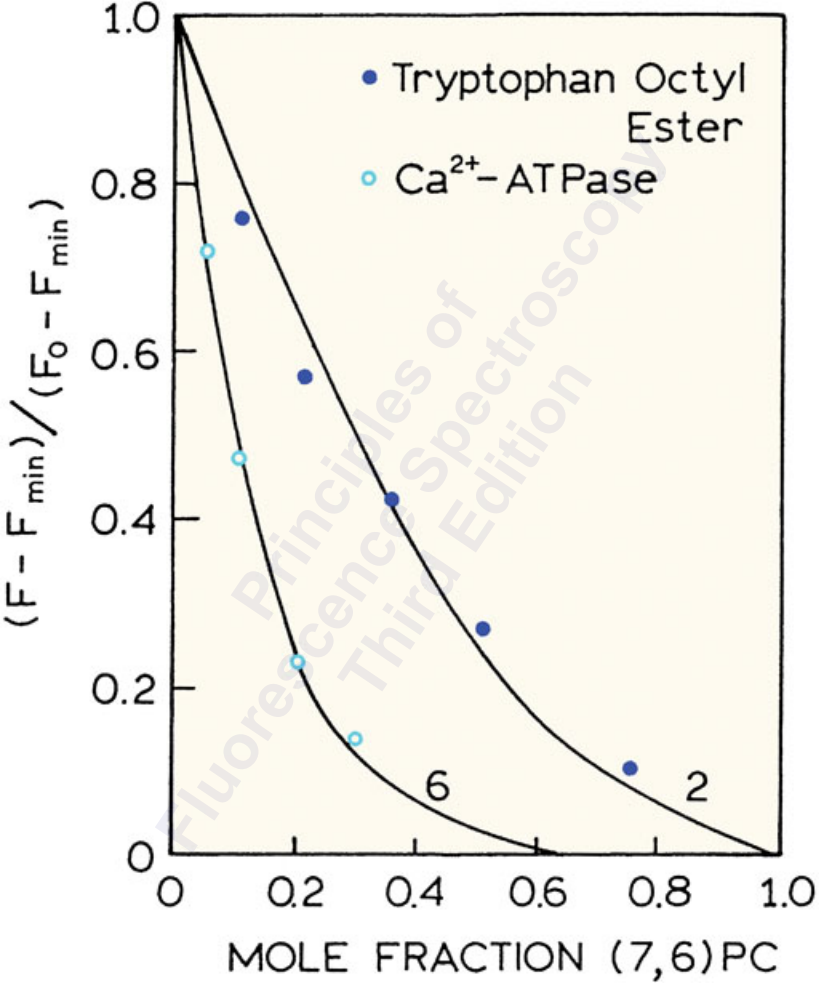


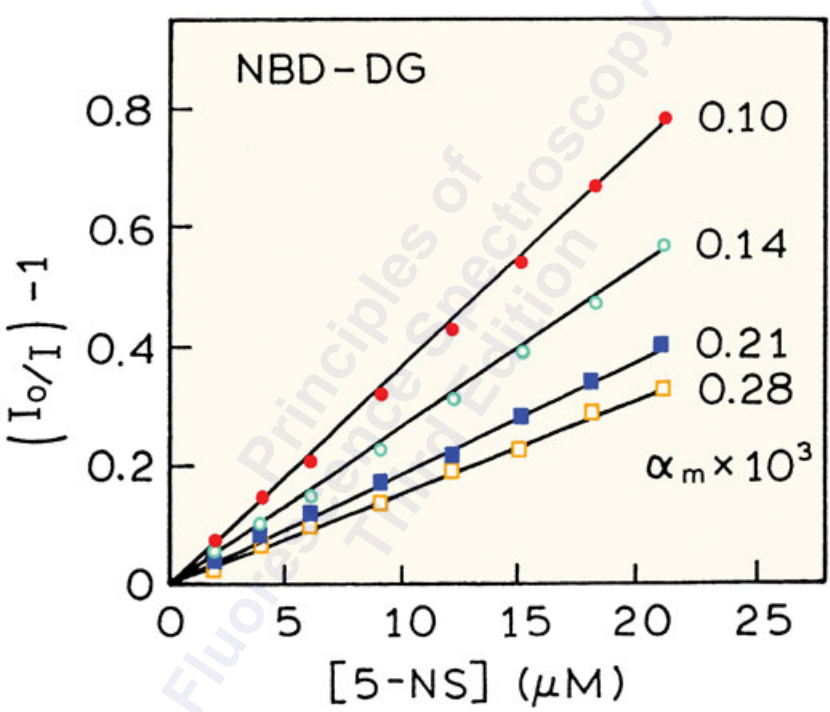


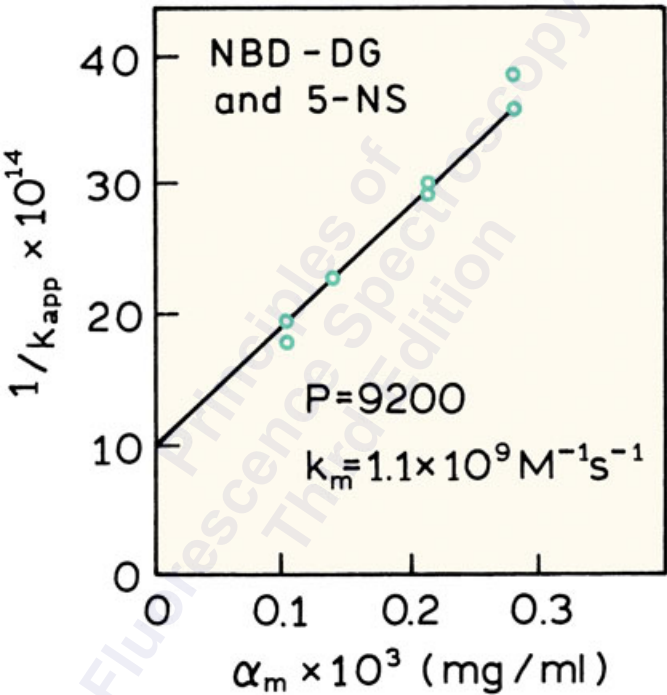




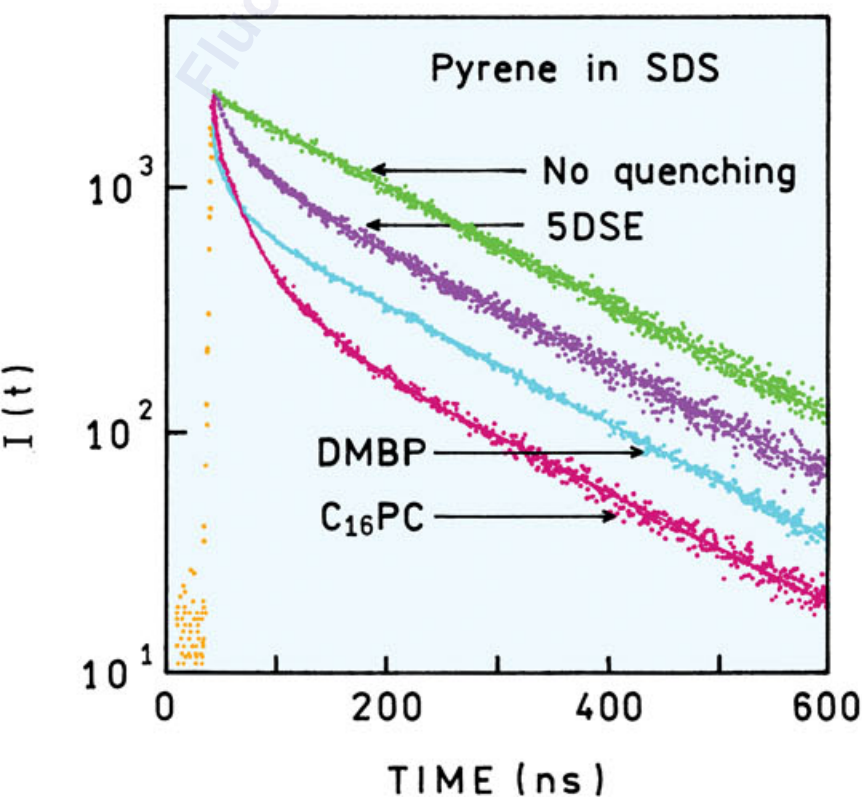
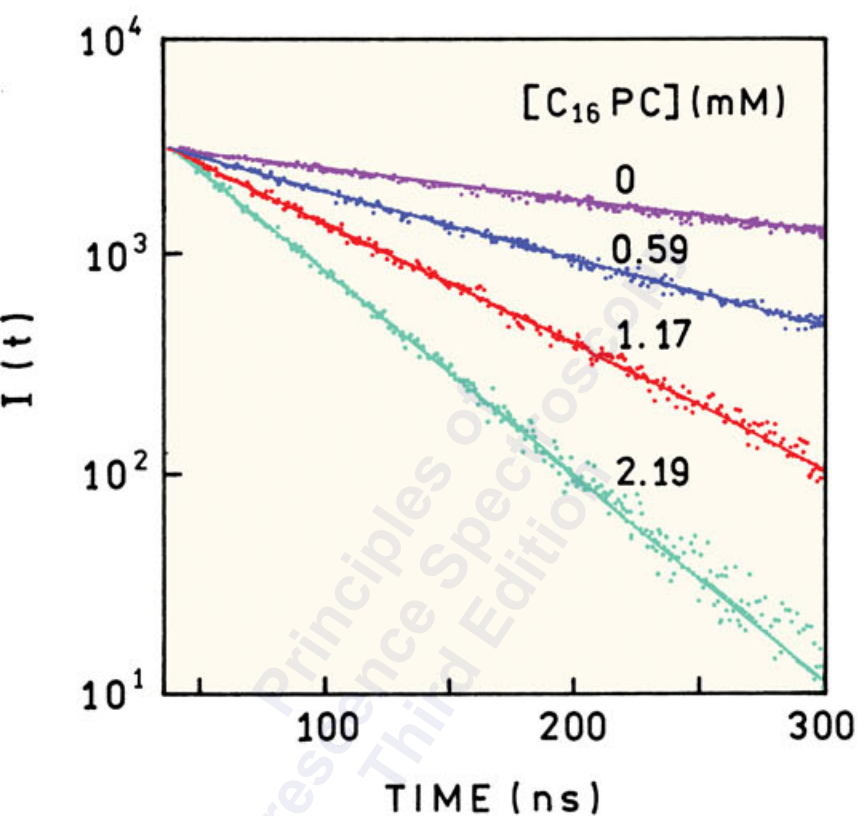
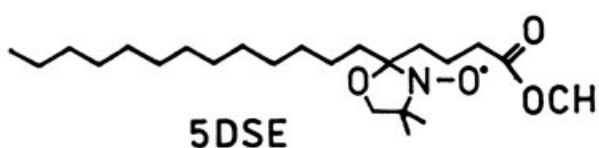
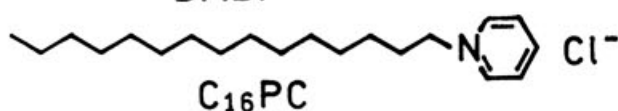
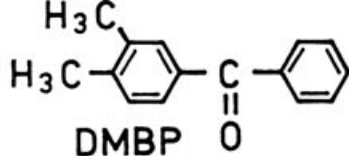


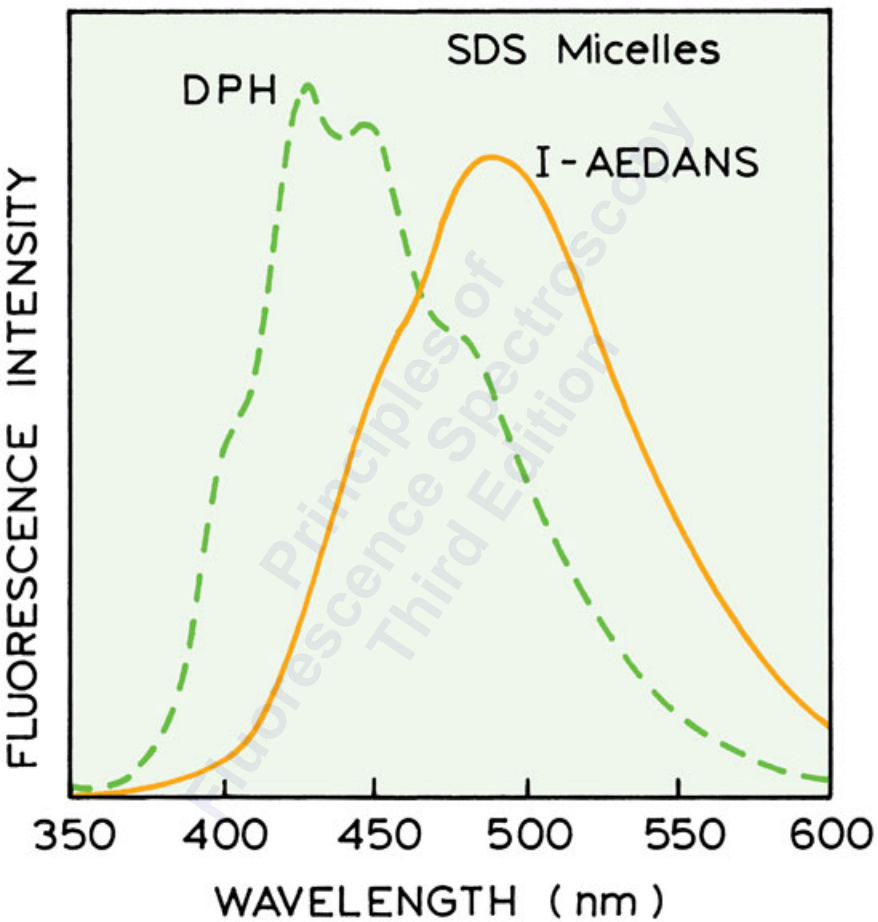


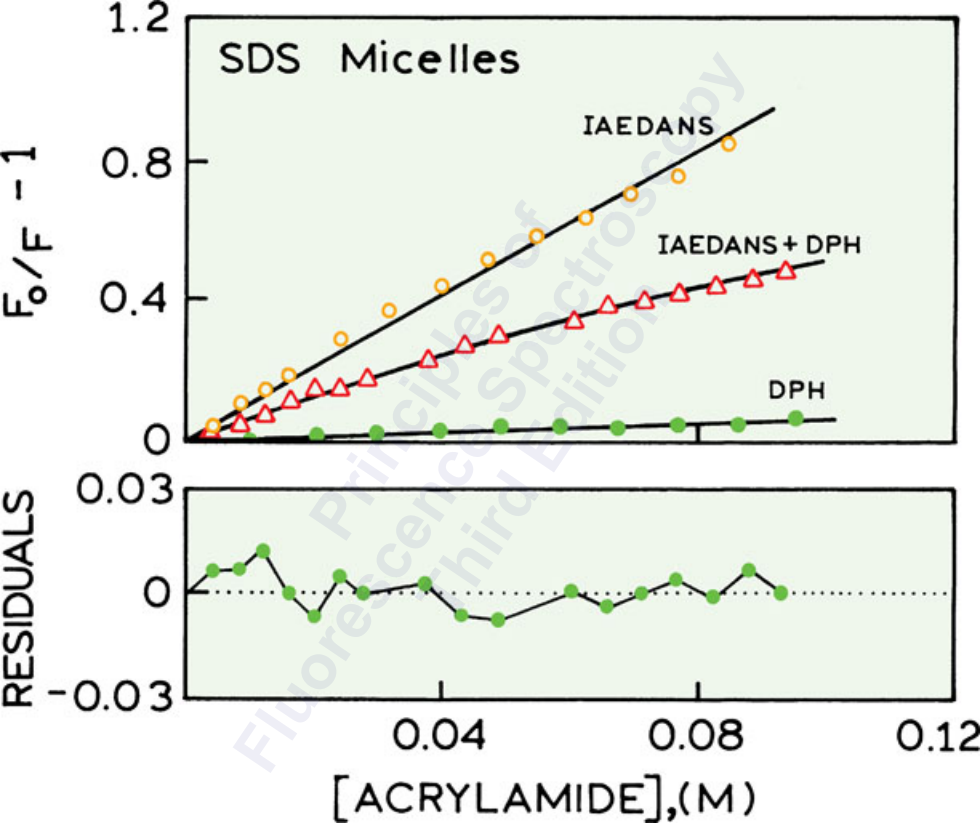


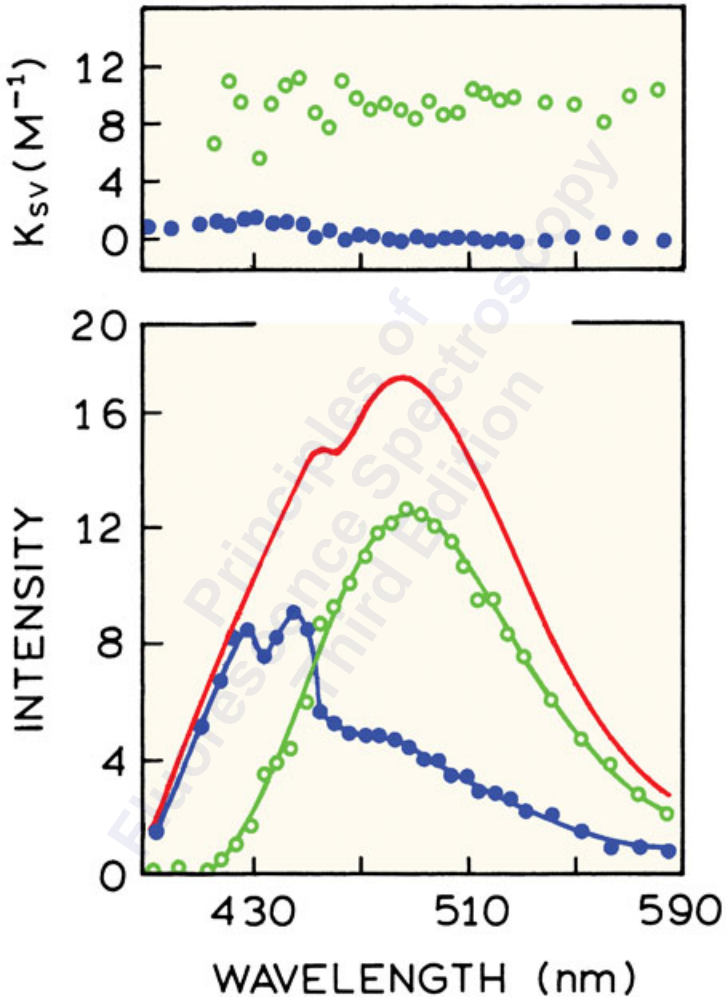


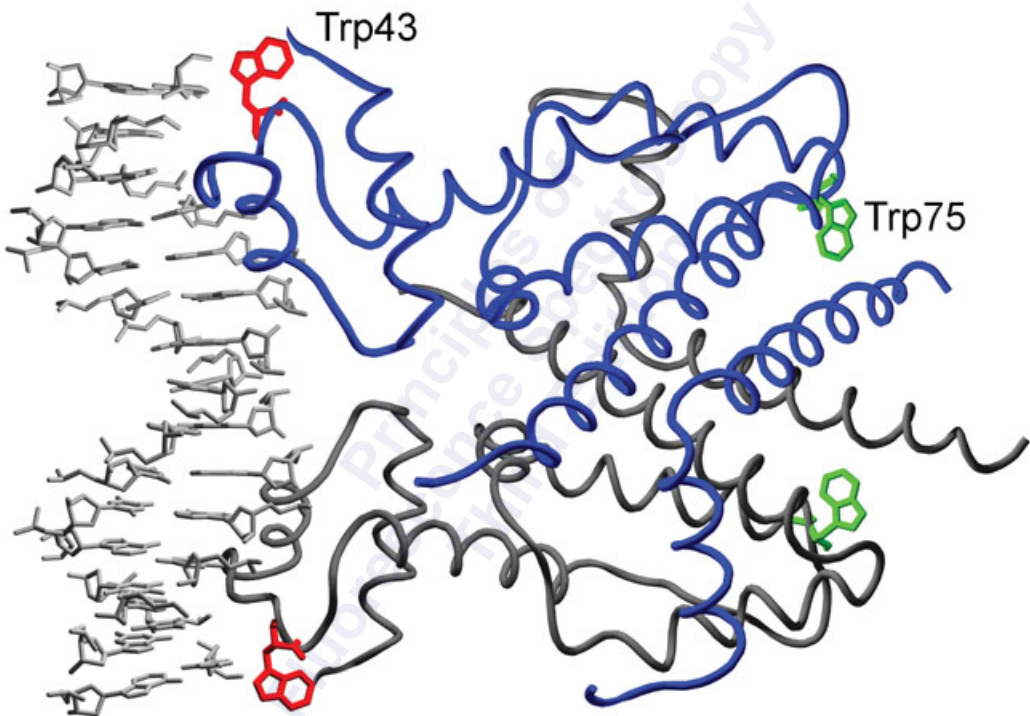


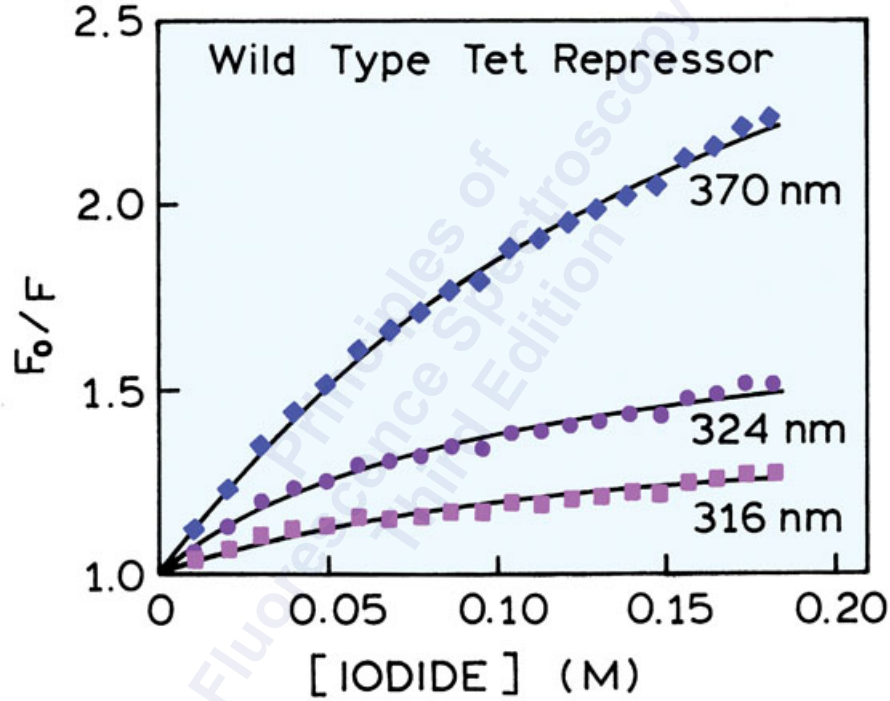


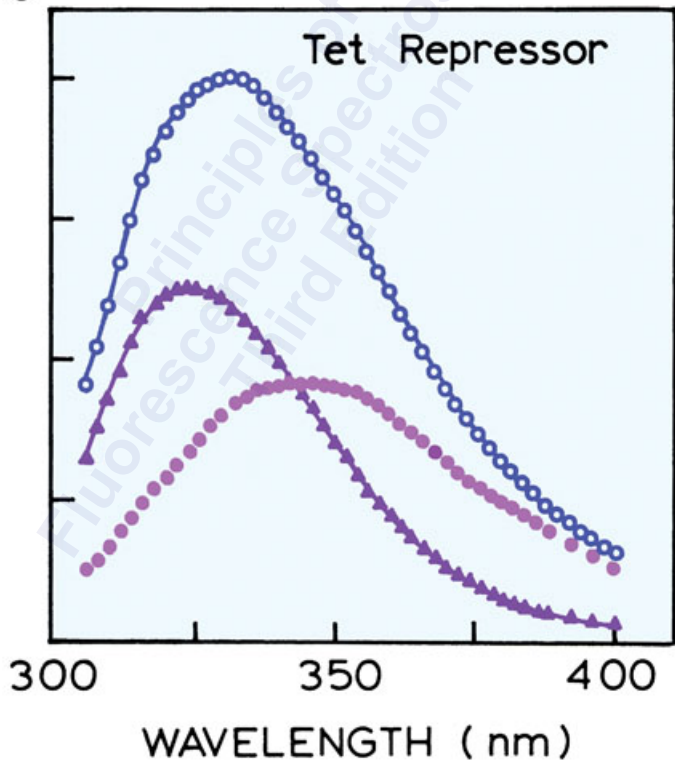
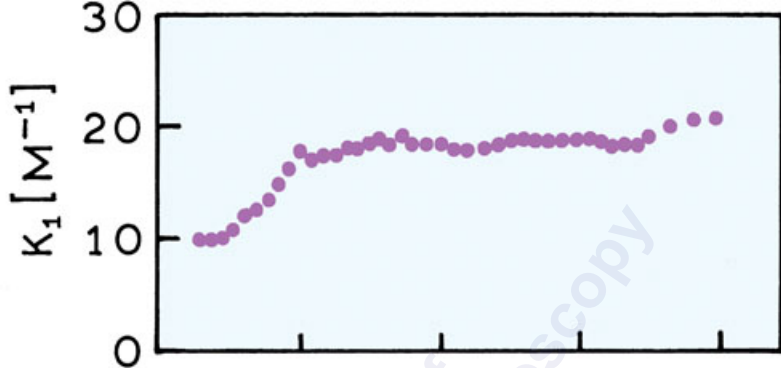


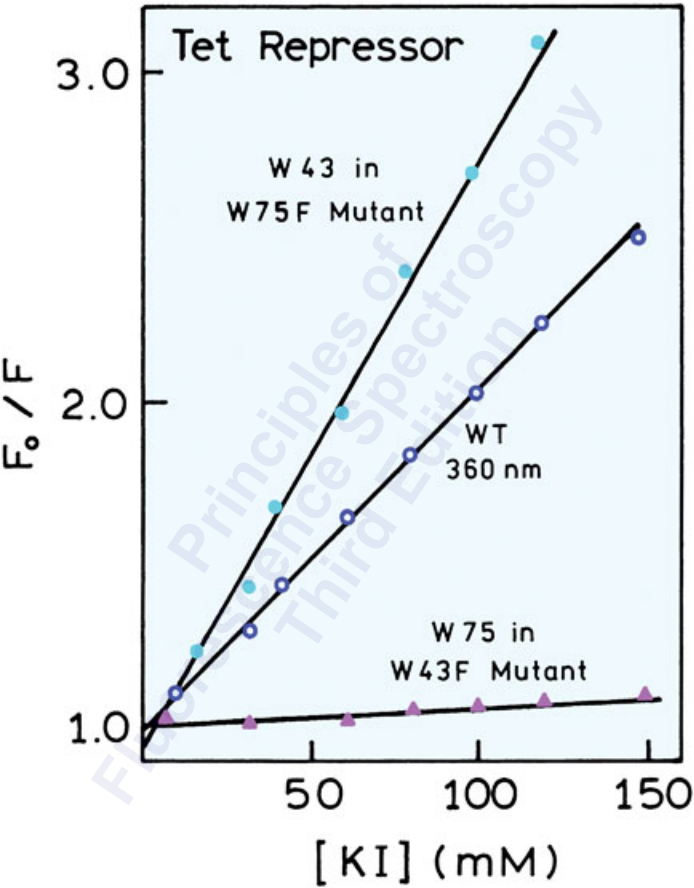




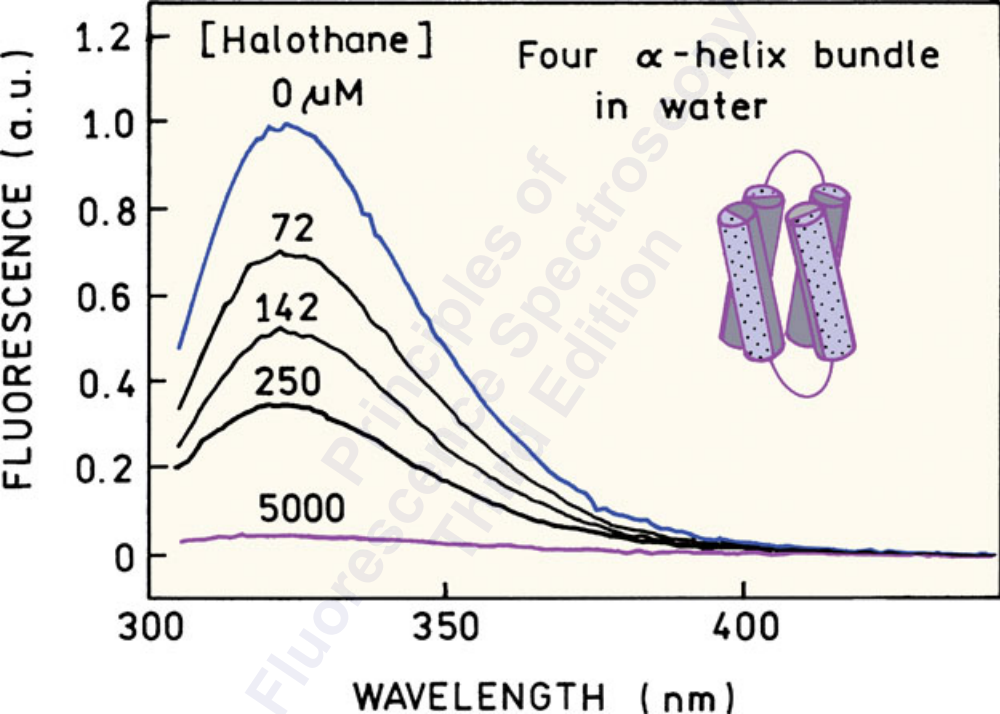












FLUORESCENCE

1.0

0.8

0.6

0.4

0.2

0

Water

0

2

4

6

8

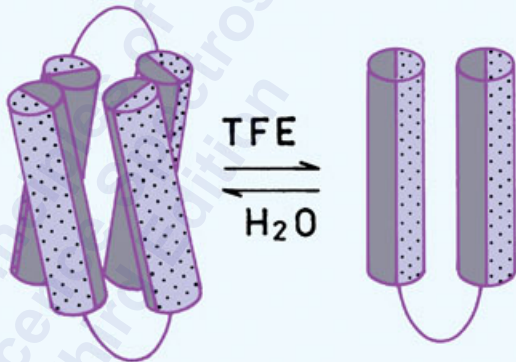
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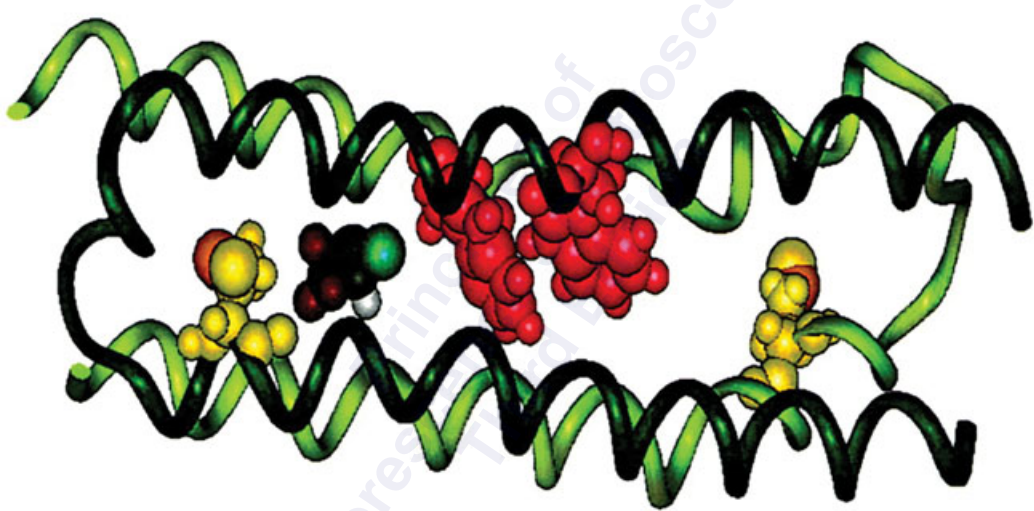
[HALOTHANE] (mM)

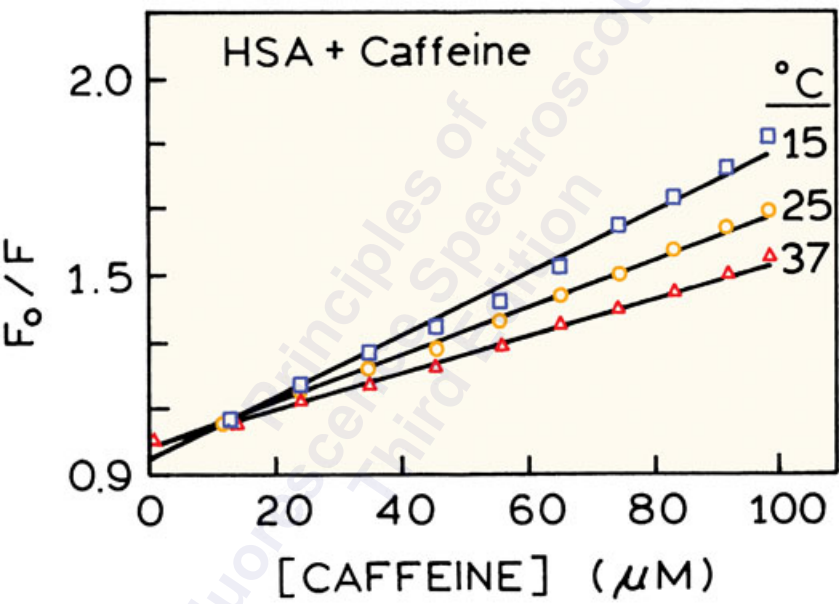
50% TFE

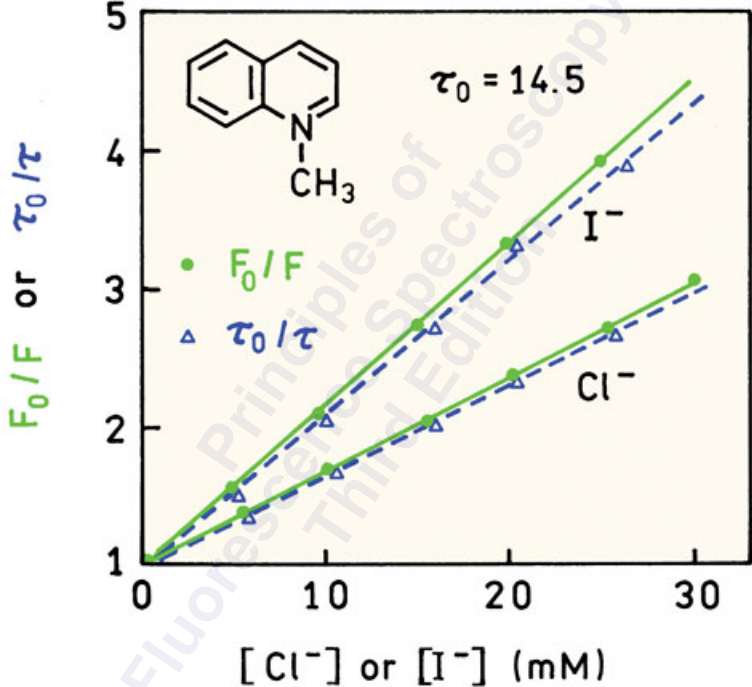
TFE

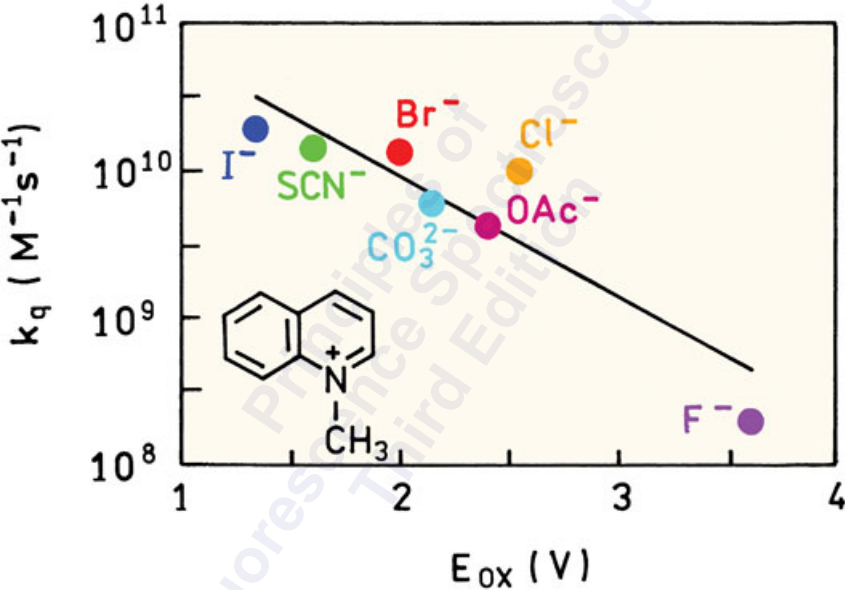
$\text{H}_2\text{O}$

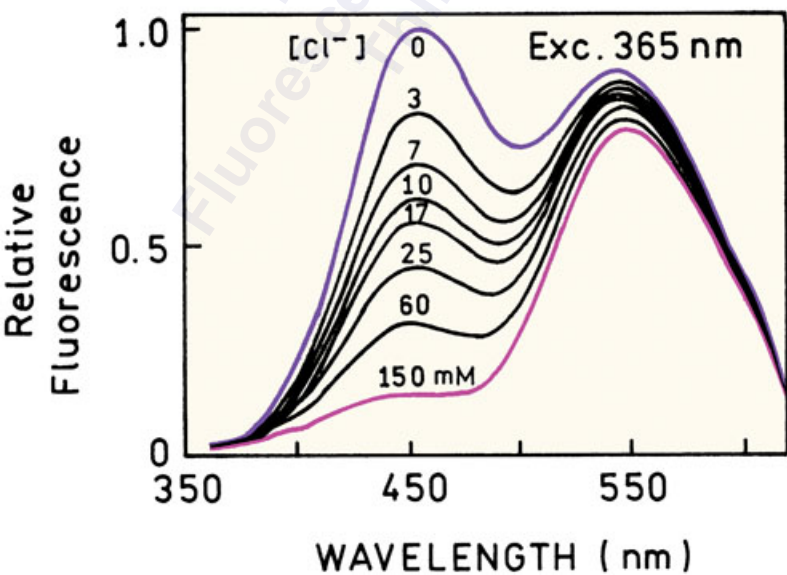
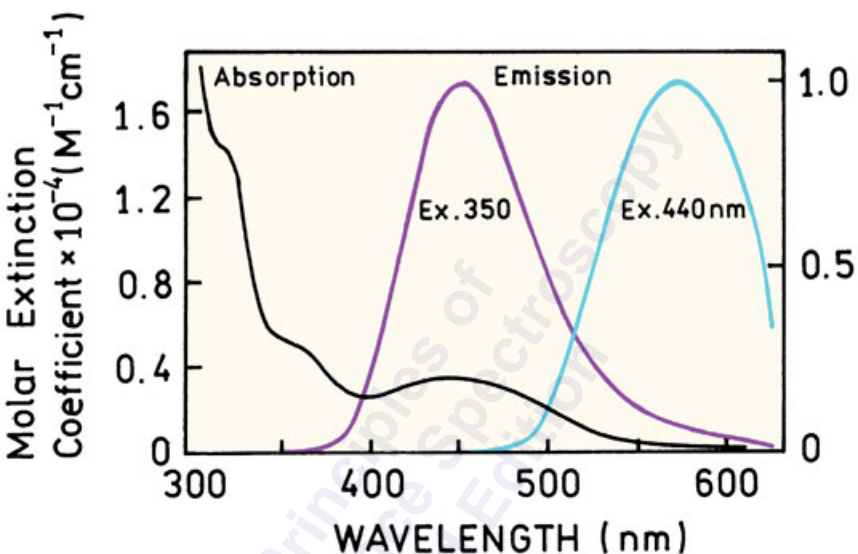
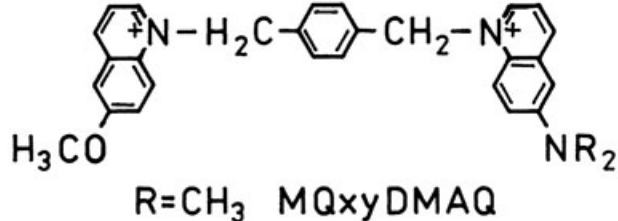


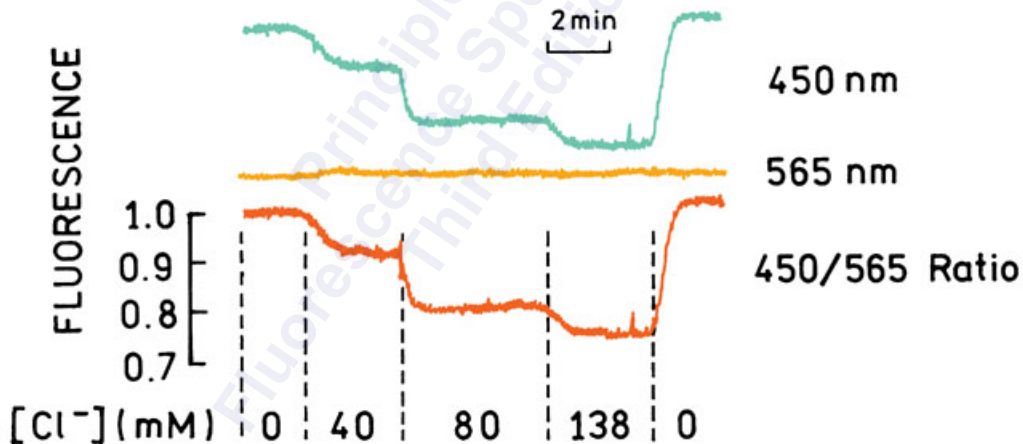
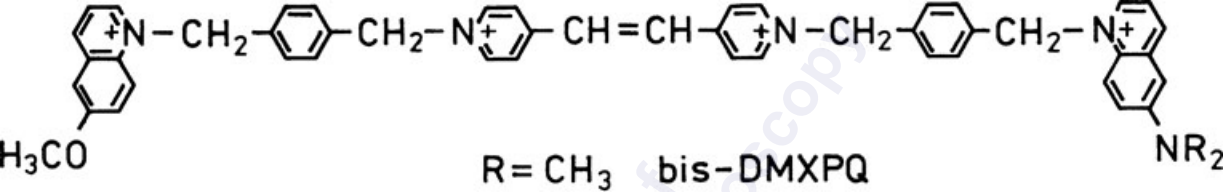




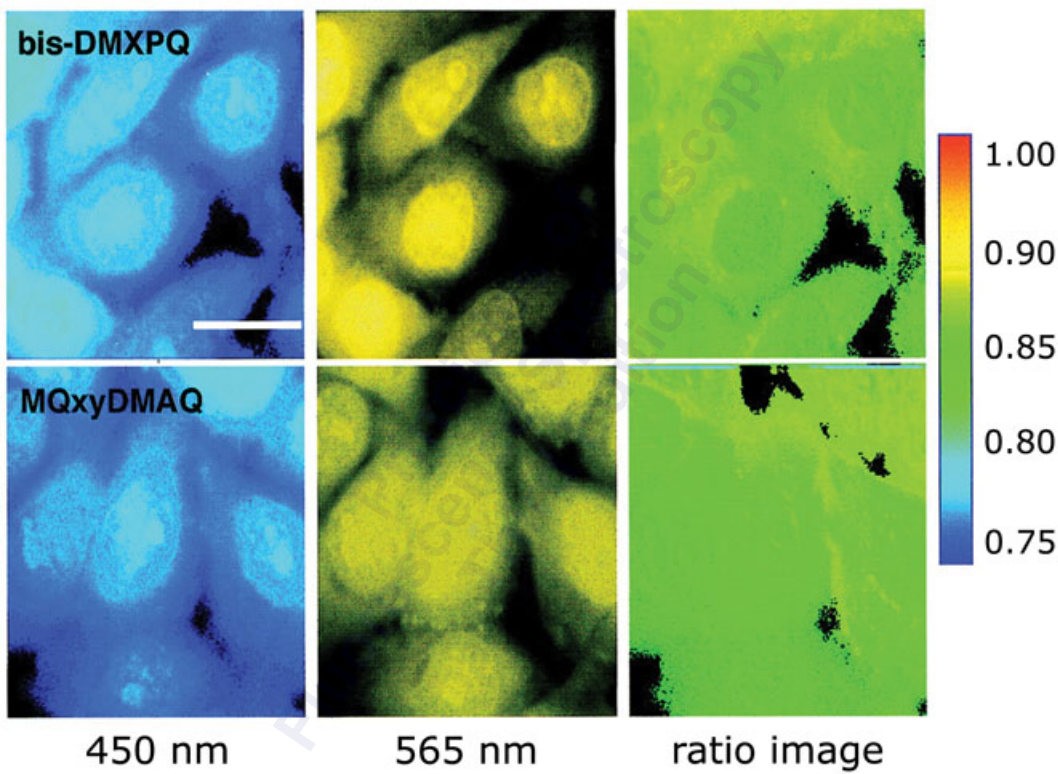


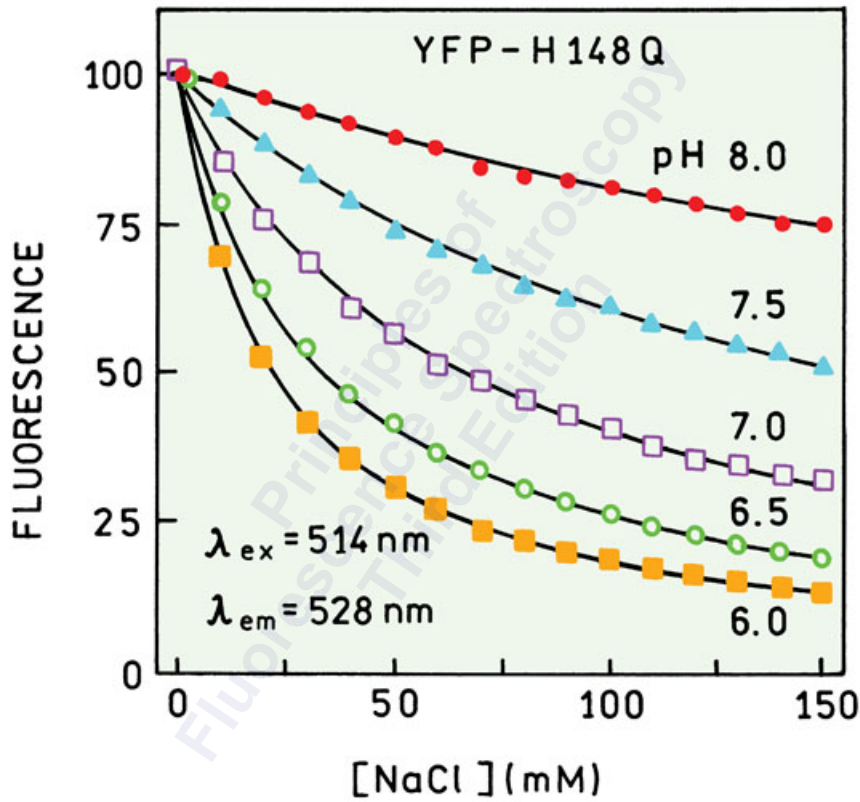


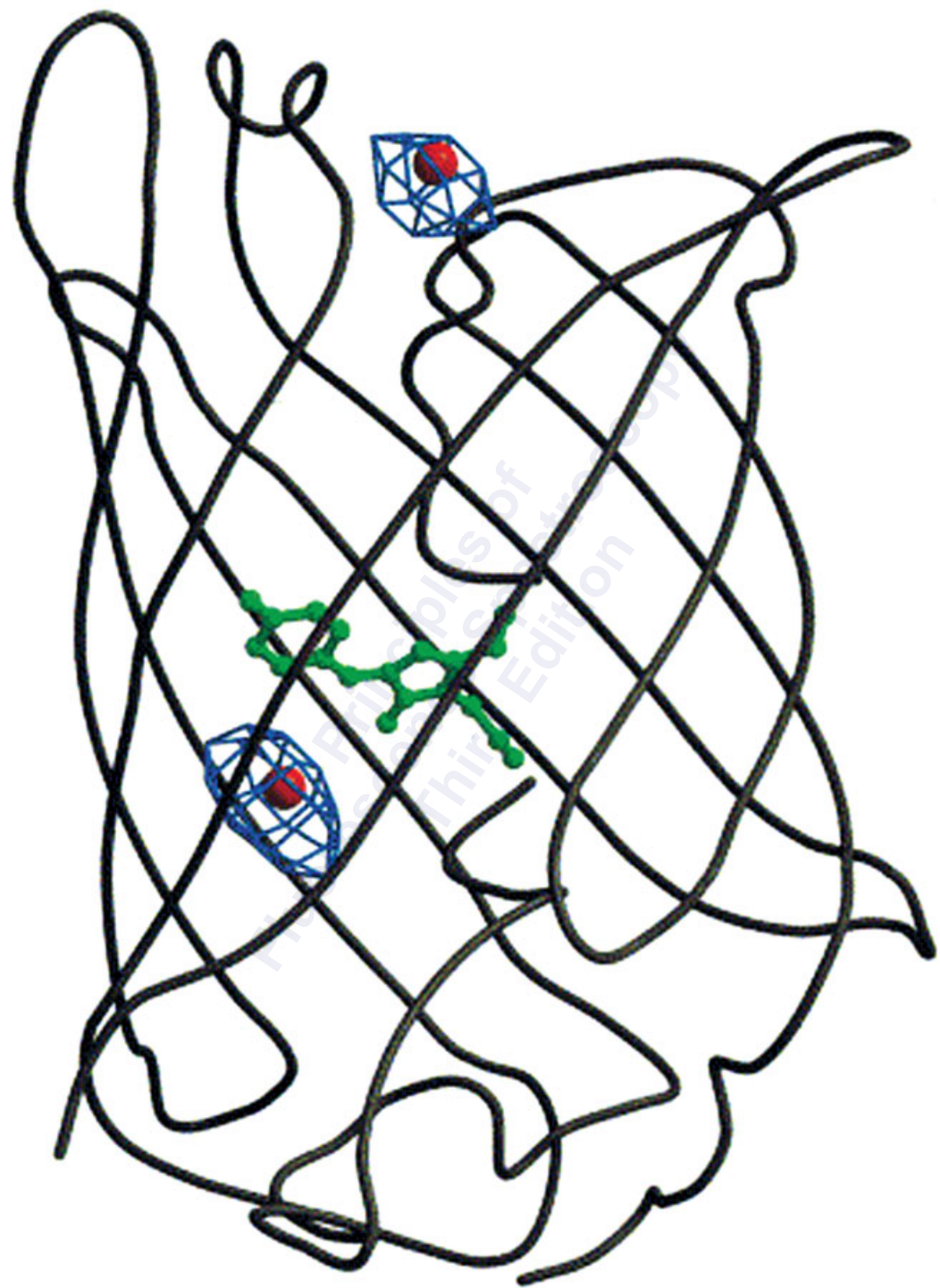


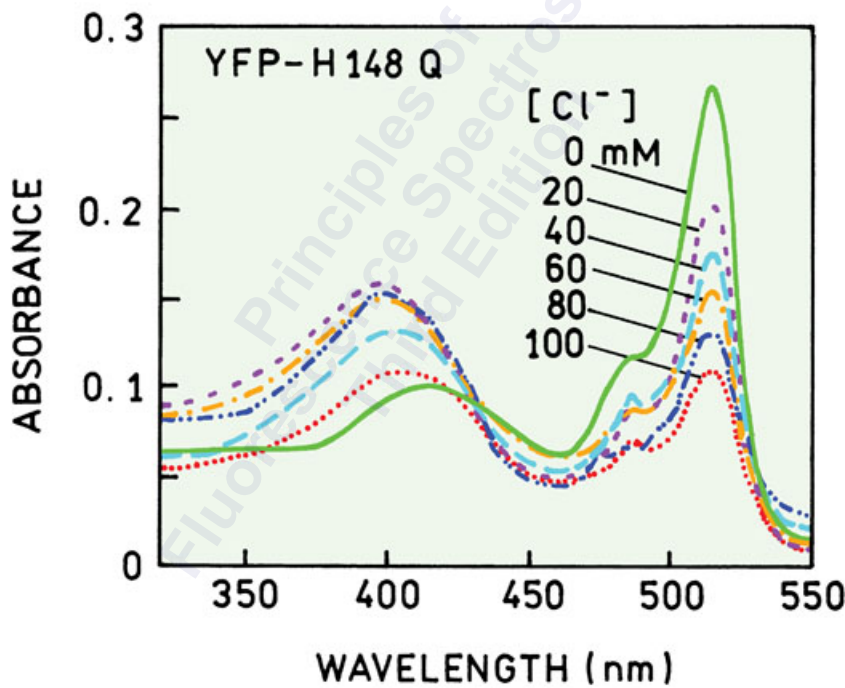
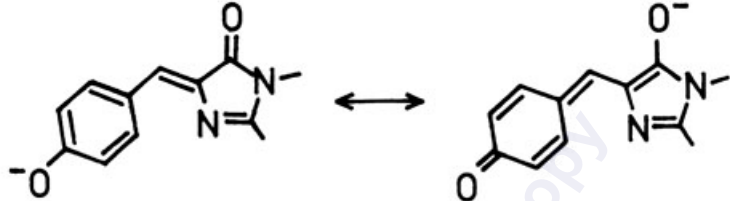


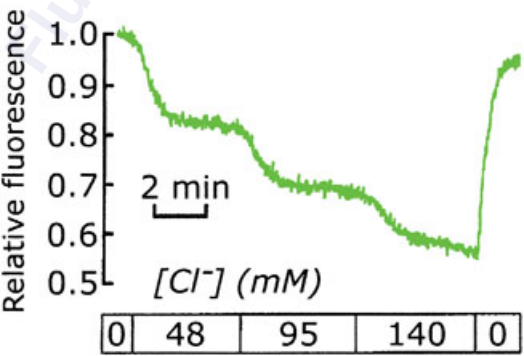
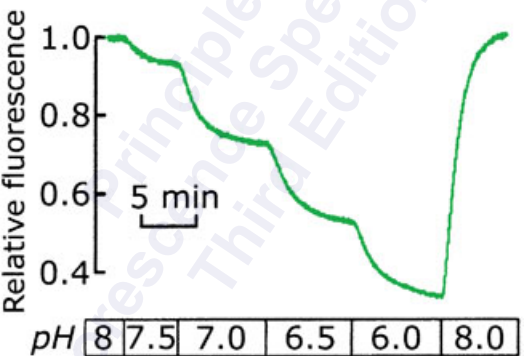
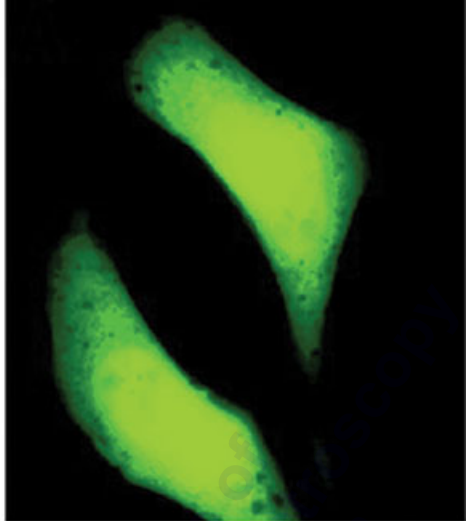


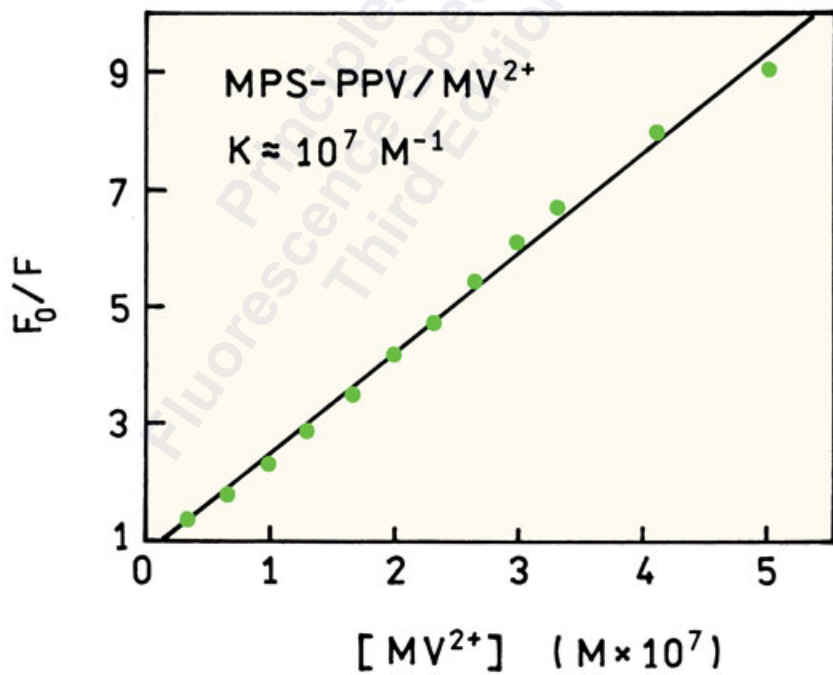
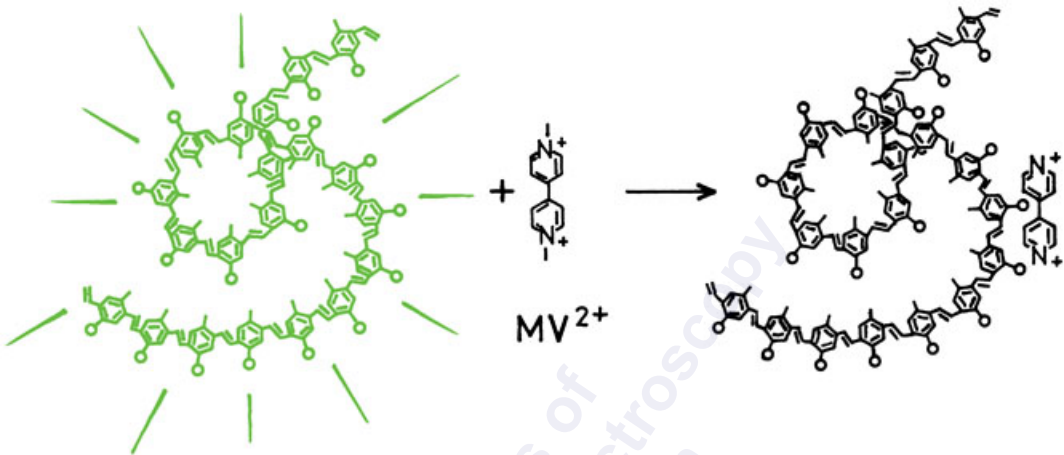


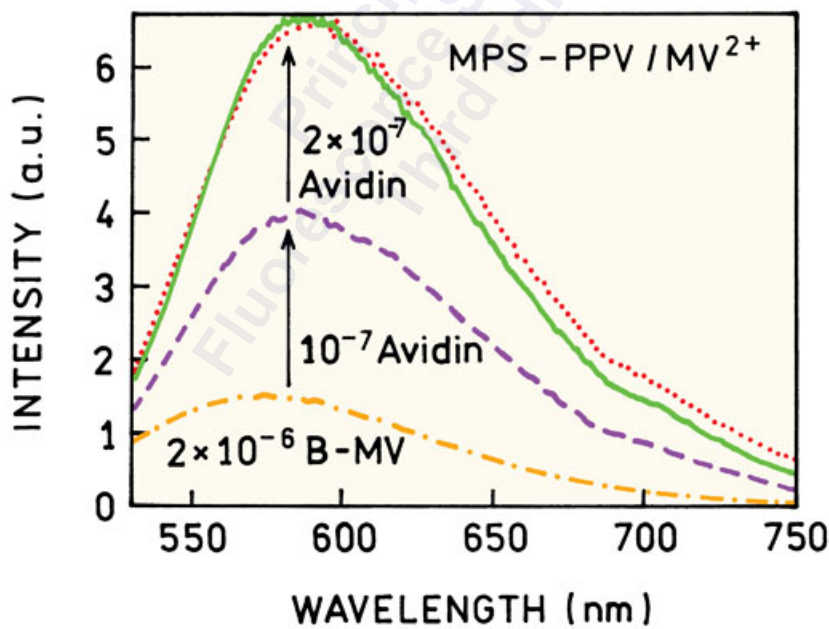
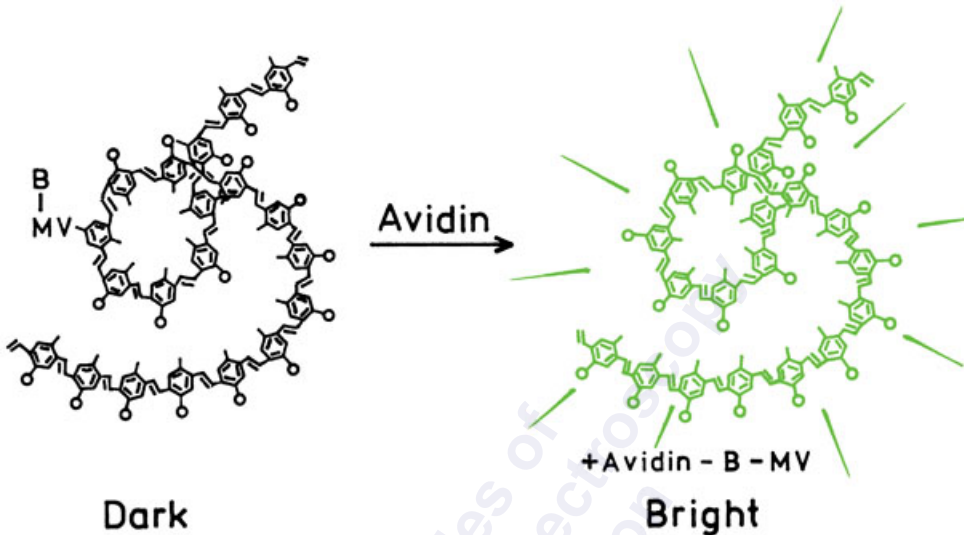




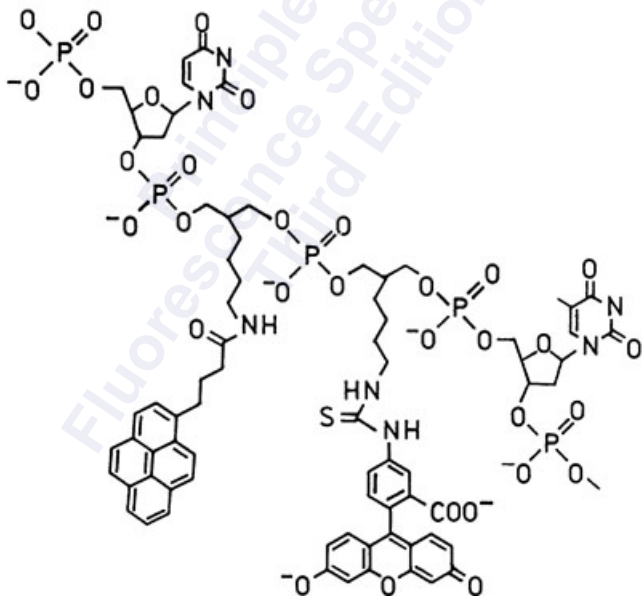
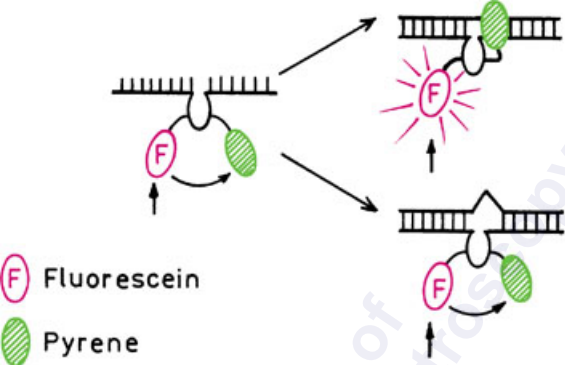




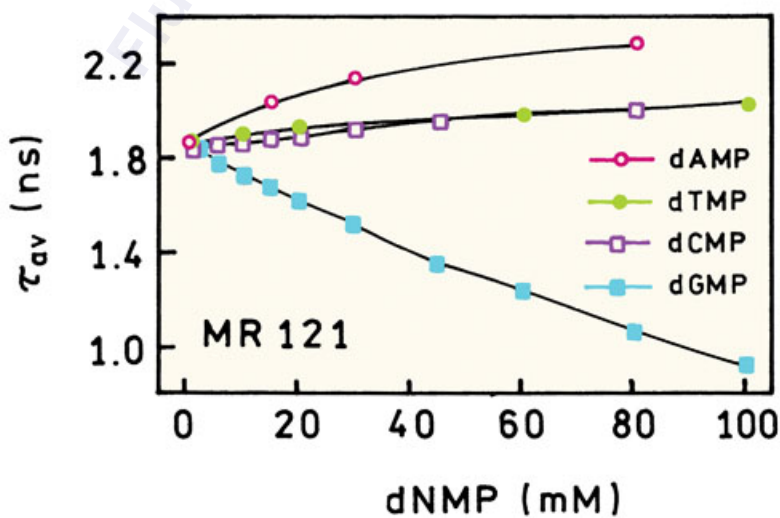
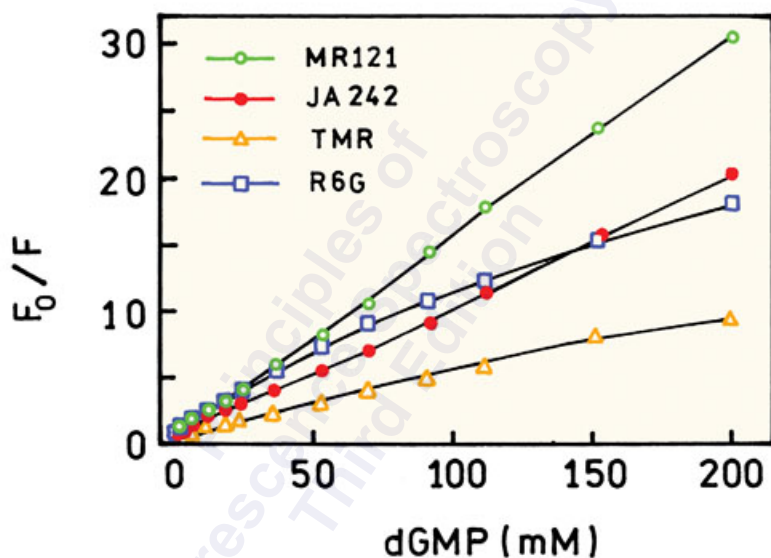
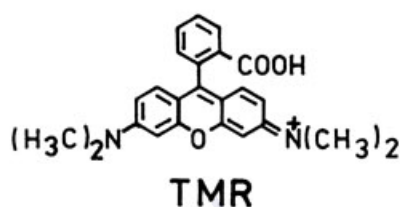
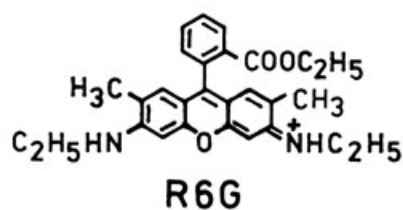
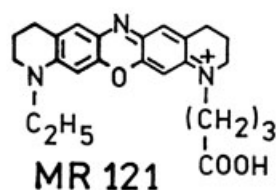
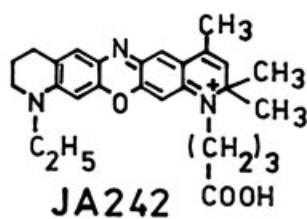


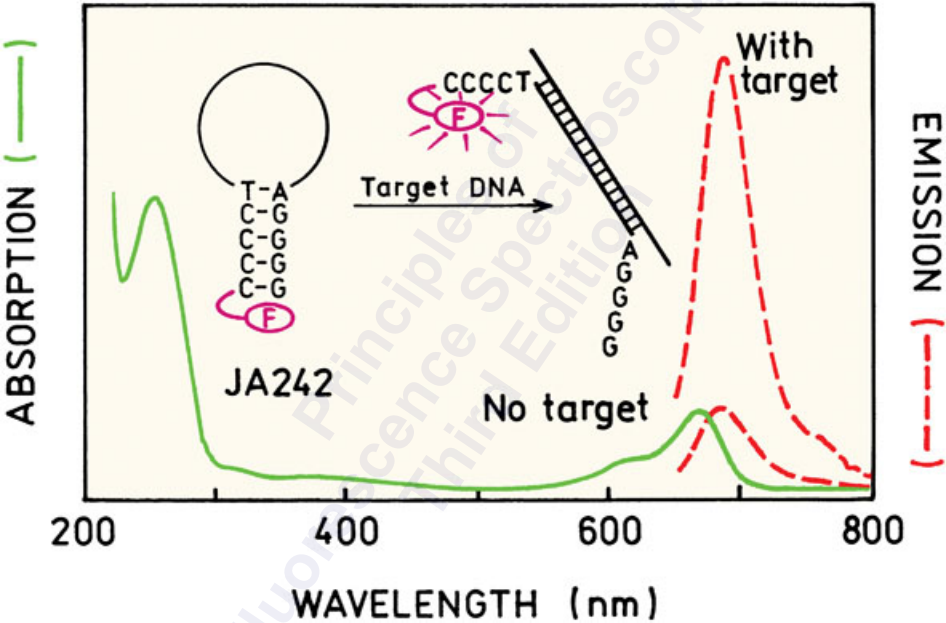




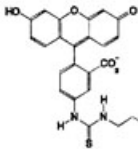








## Fluorescein

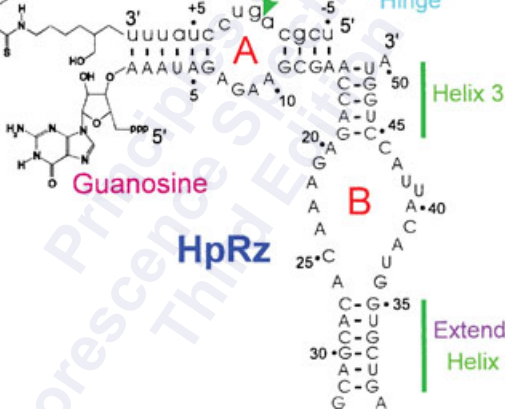


## Substrate

Helix 1

## Helix 2

## "Hinge"

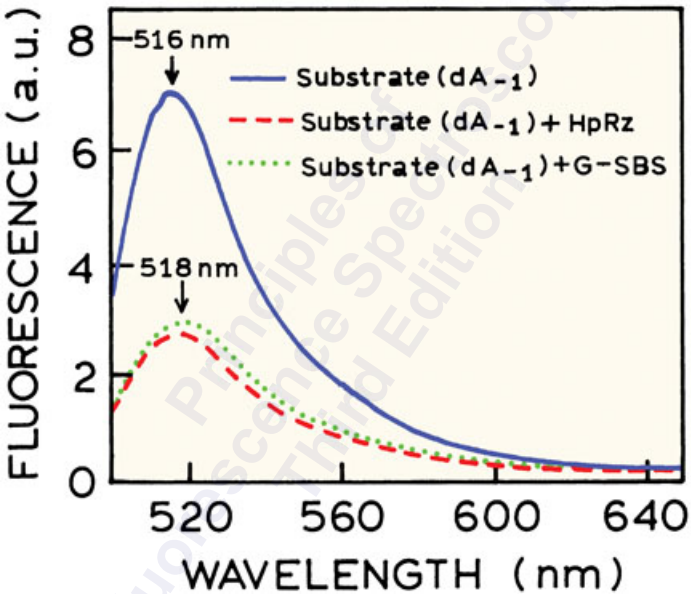


## Guanosine

HpRz

### Helix 3

Extended  
Helix 4



$\epsilon$  ADP and  
Dna B Helicase

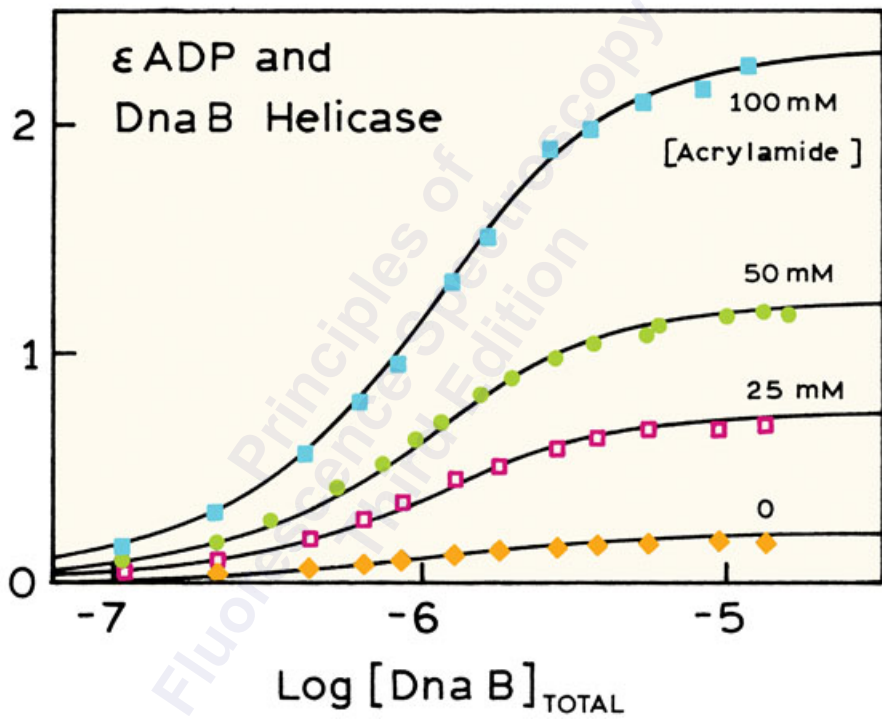
100 mM  
[Acrylamide]

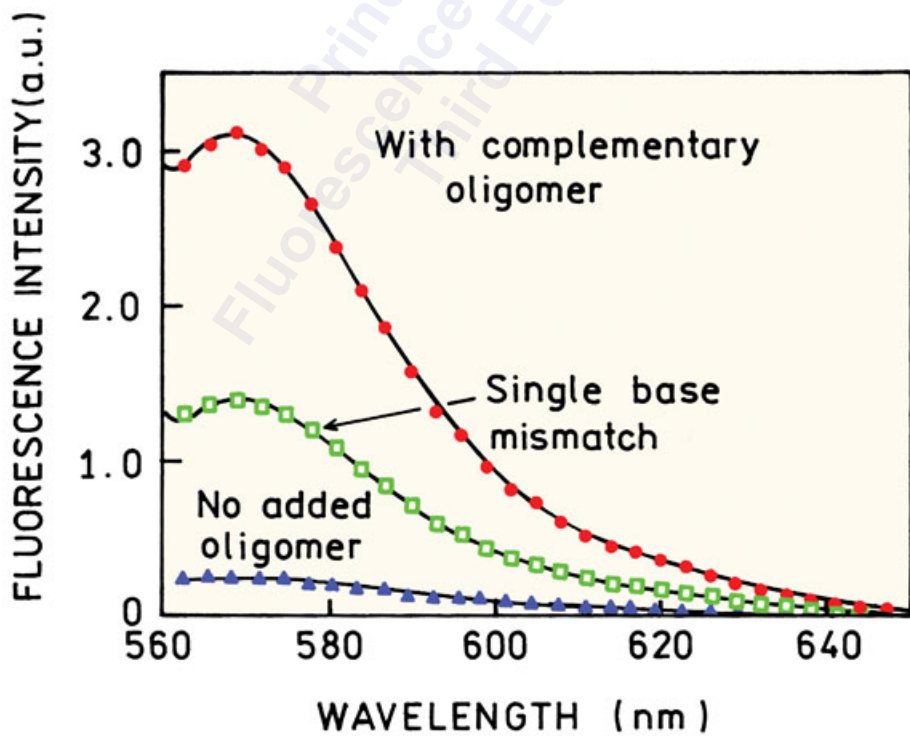
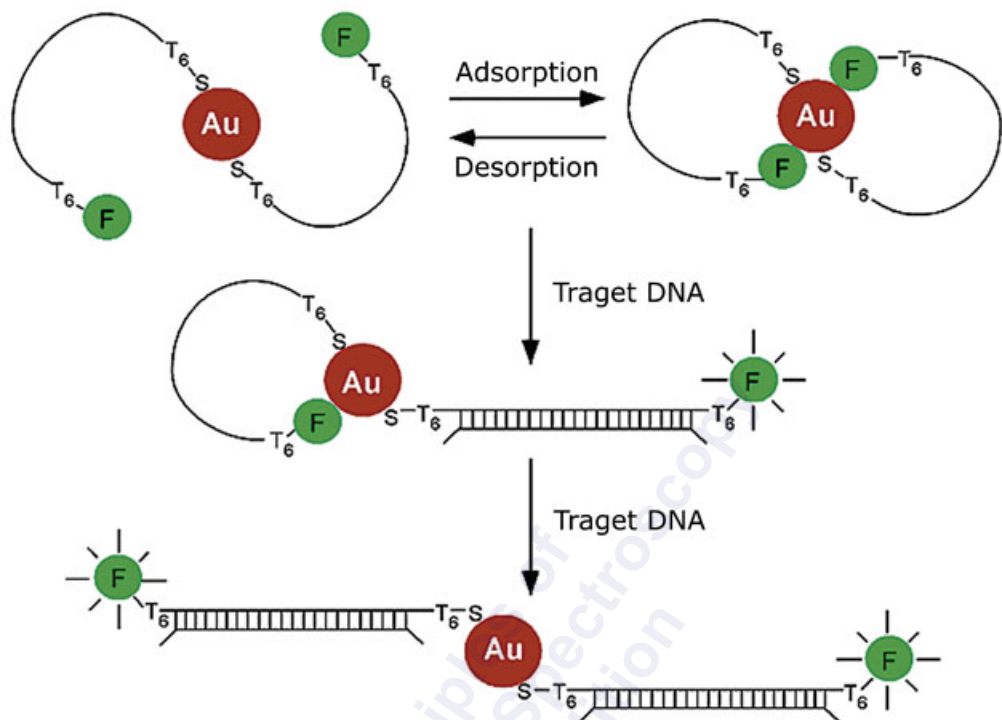
50 mM

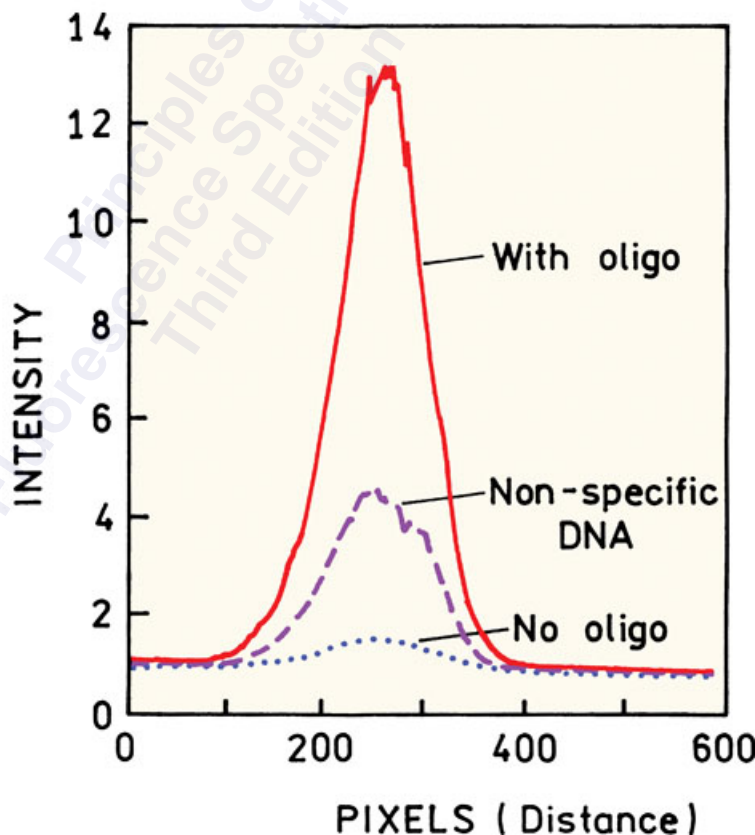
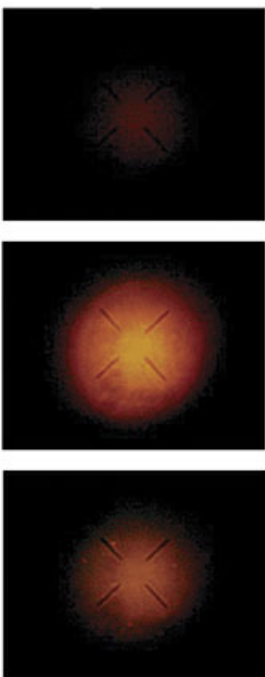
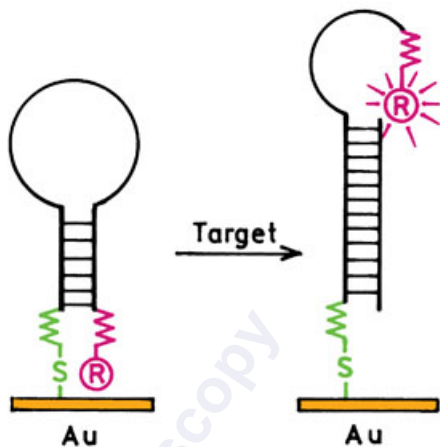
25 mM

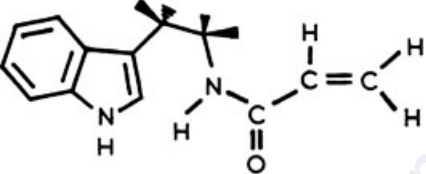
0

Log [Dna B]<sub>TOTAL</sub>

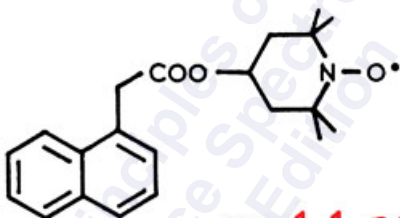




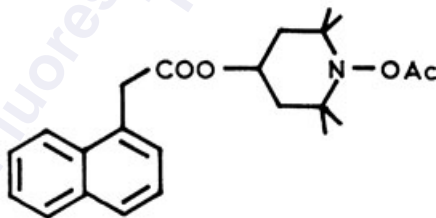




$$\tau = 31 \text{ ps}$$

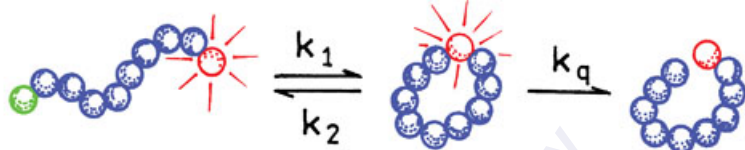


$$\tau = 1.1 \text{ ns}$$

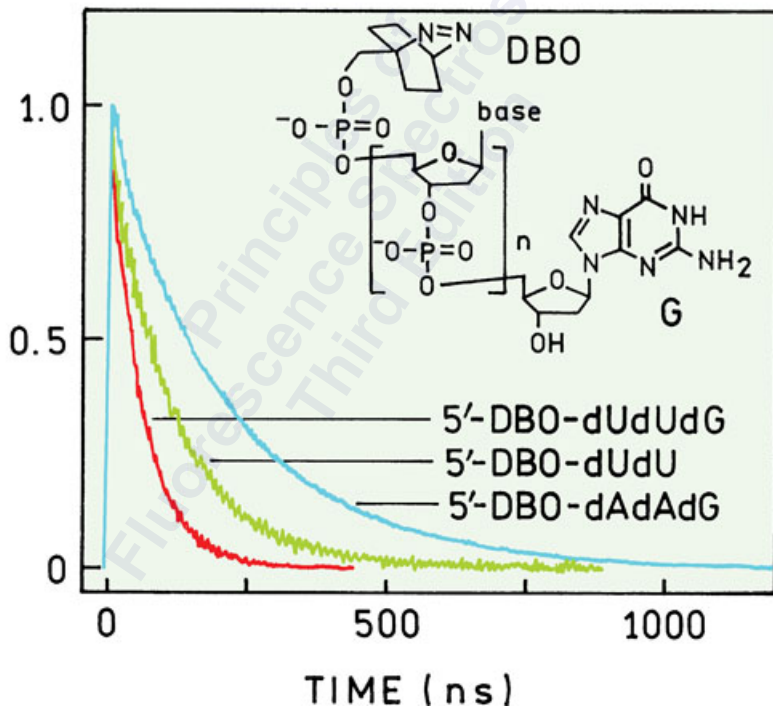


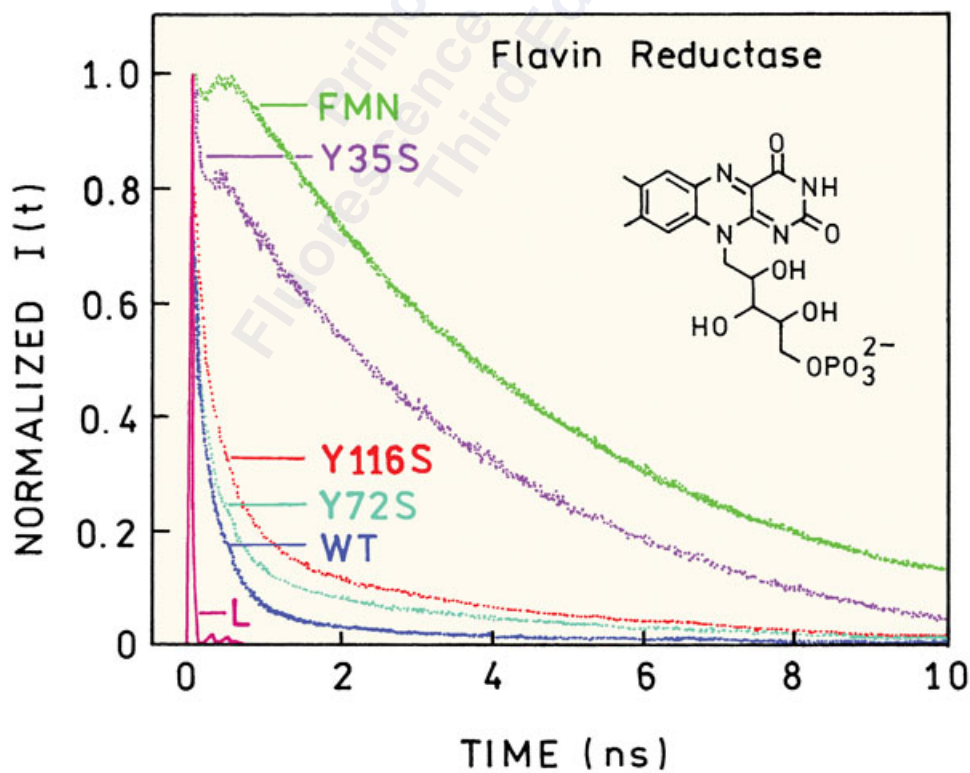
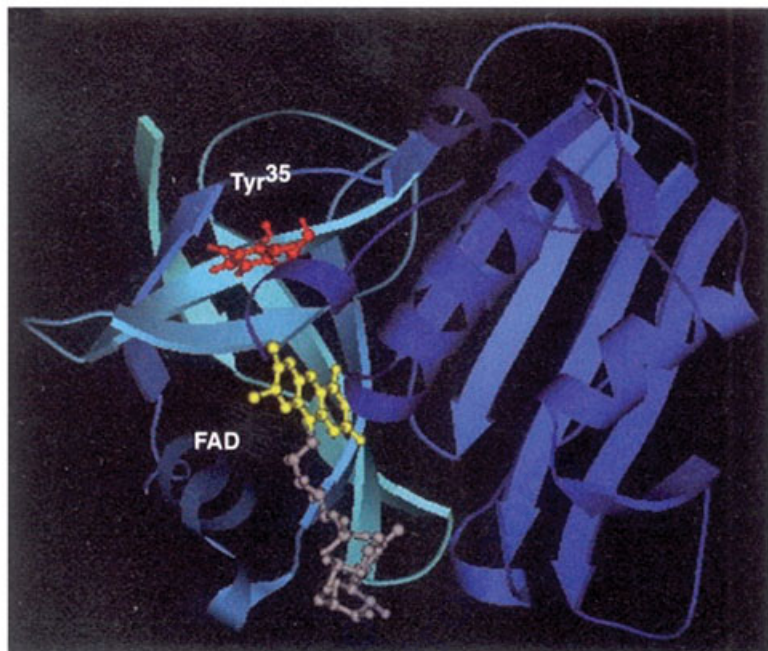
$$\tau = 33.7 \text{ ns}$$

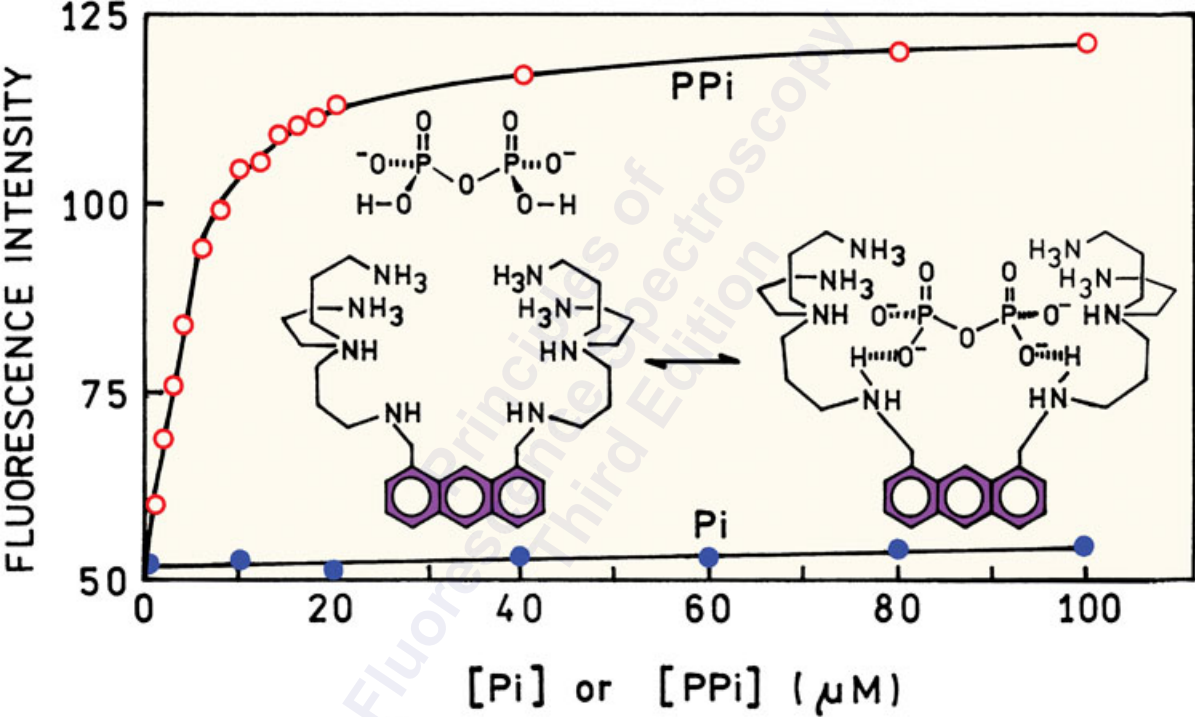


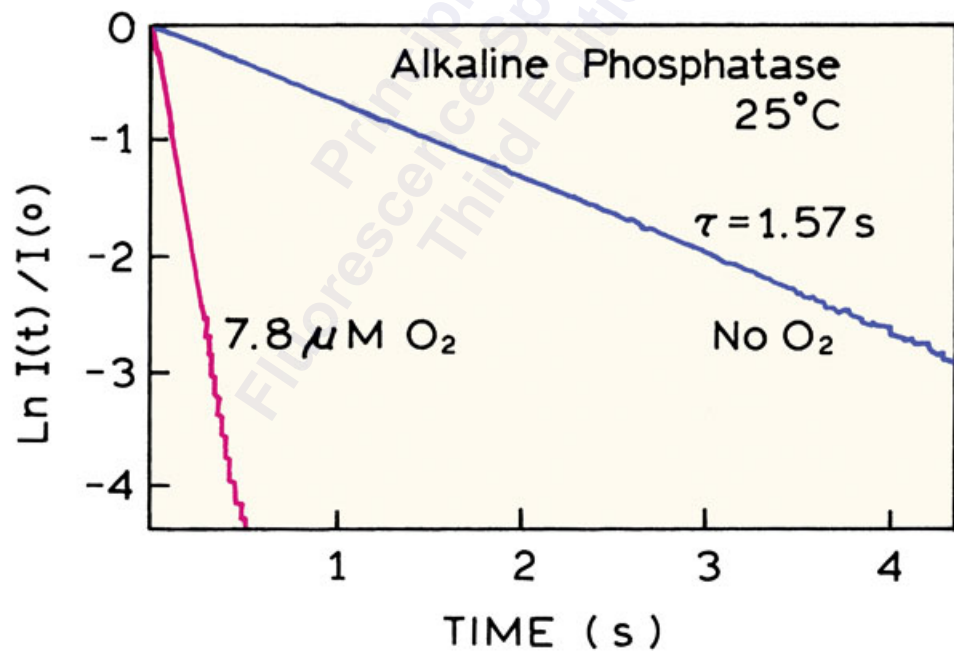
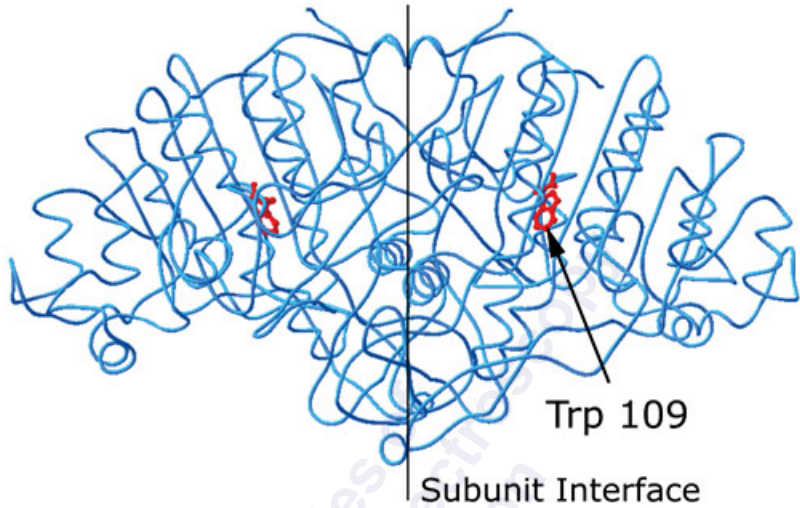


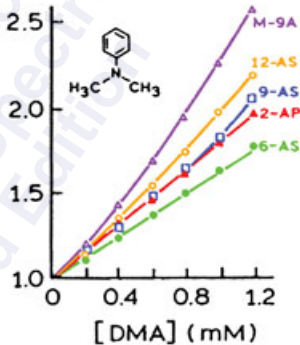
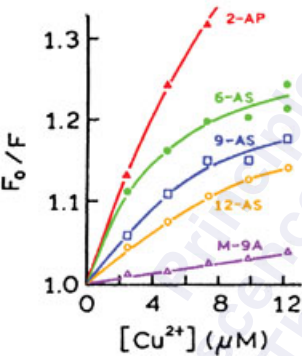
FLUORESCENCE INTENSITY

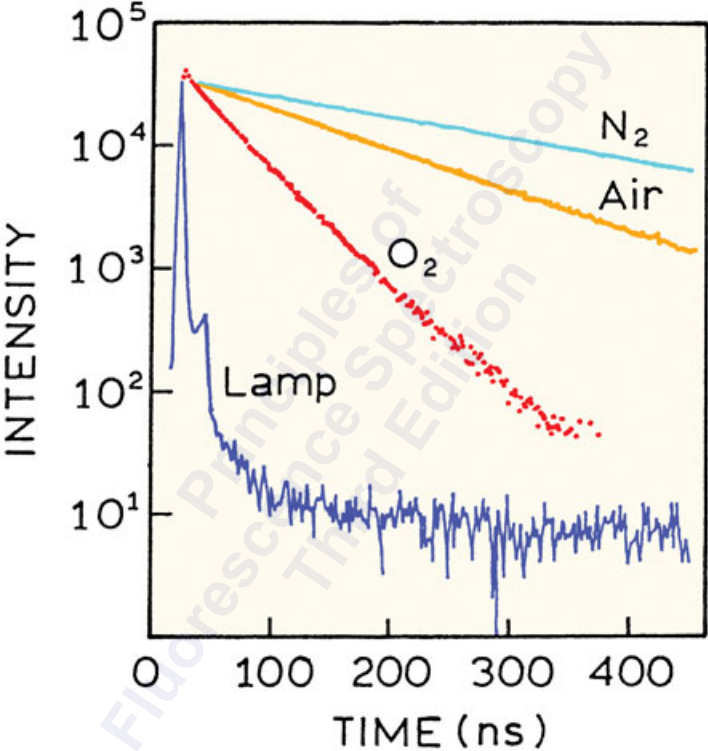


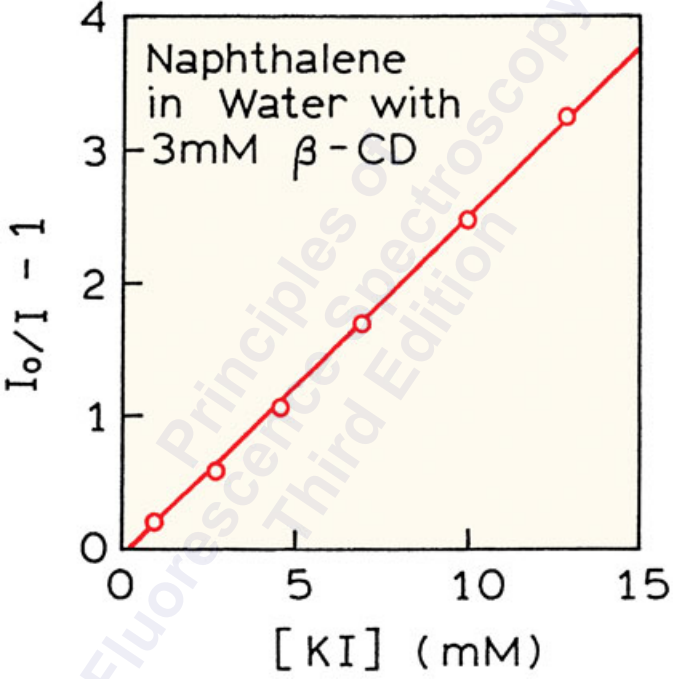


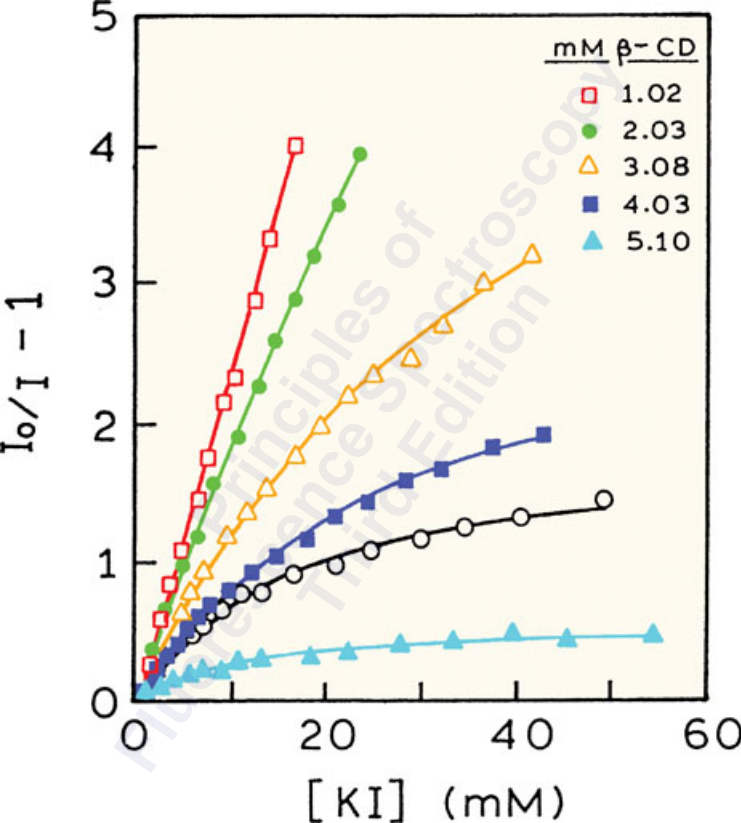




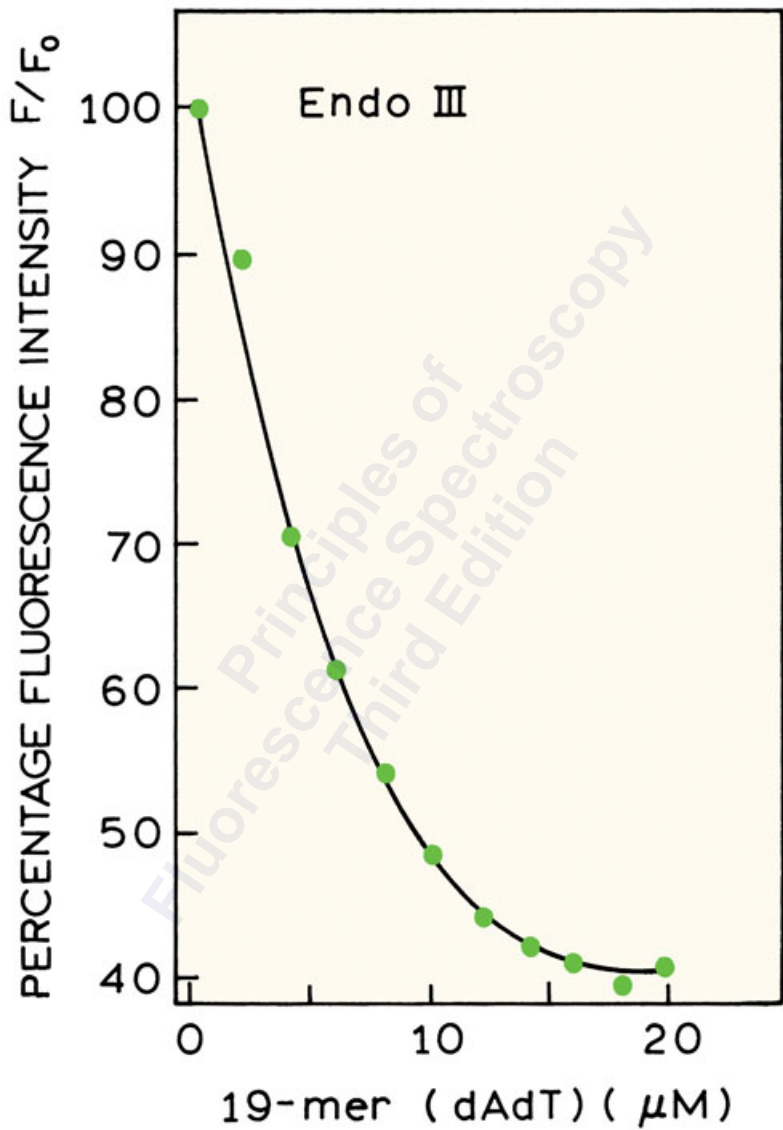




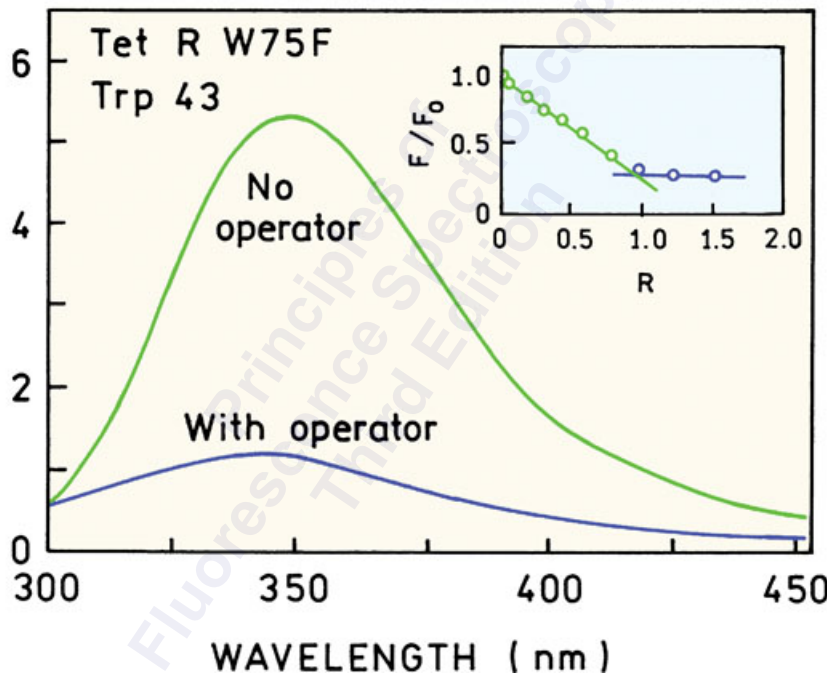


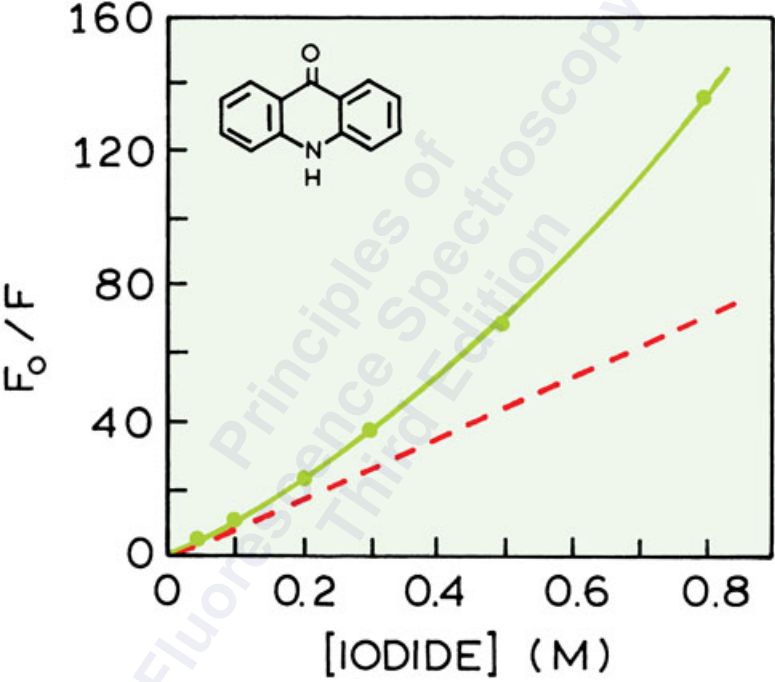


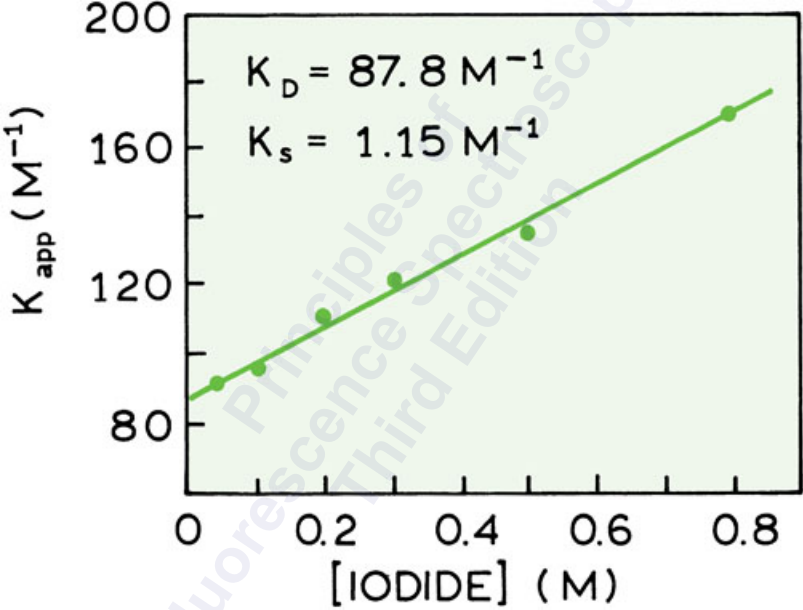


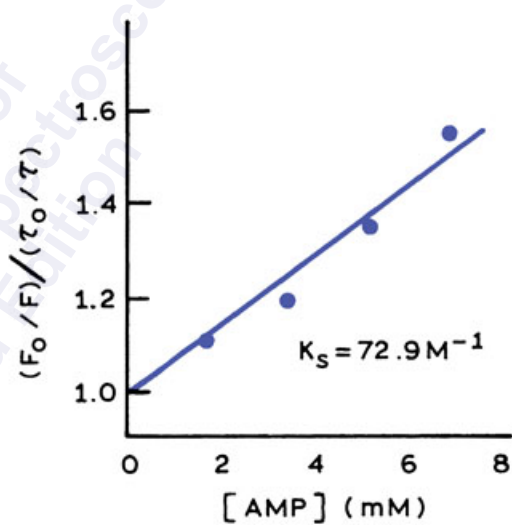
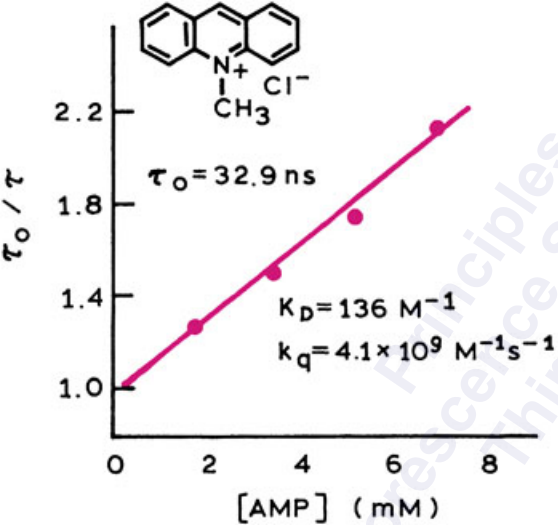


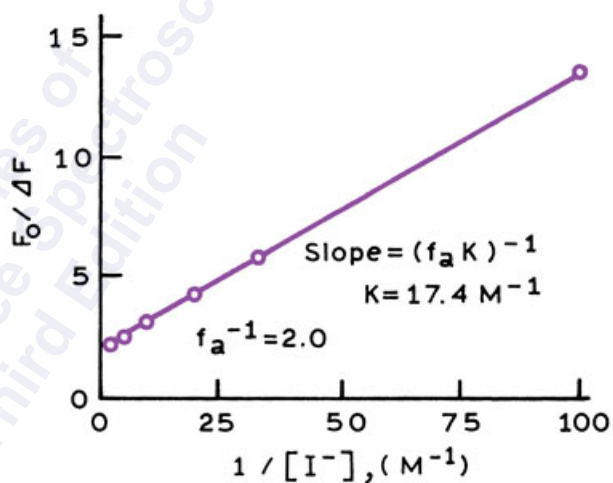
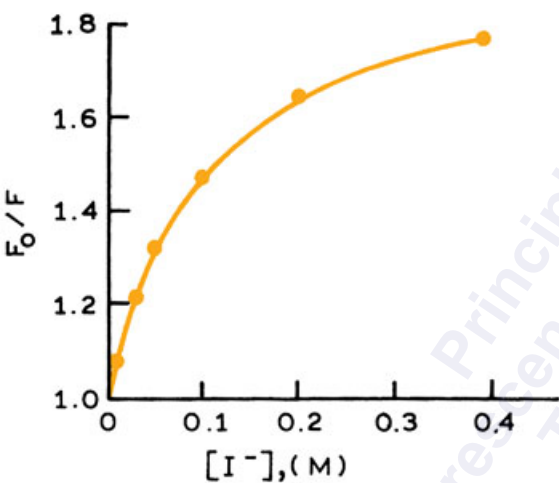
FLUORESCENCE INTENSITY (a.u.)

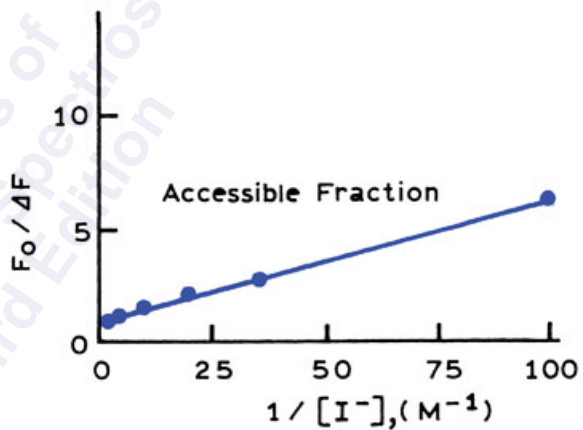
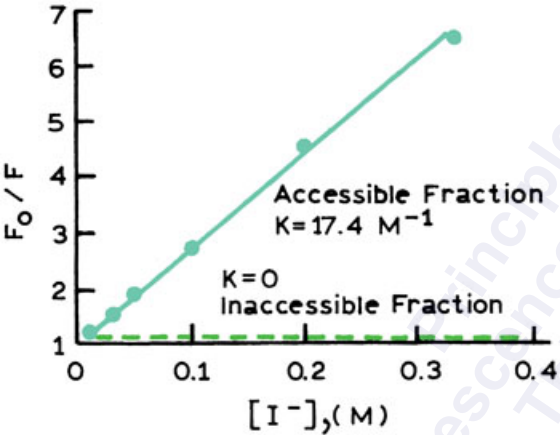






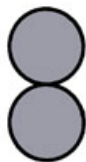
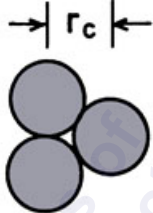












Quenching



+ heat

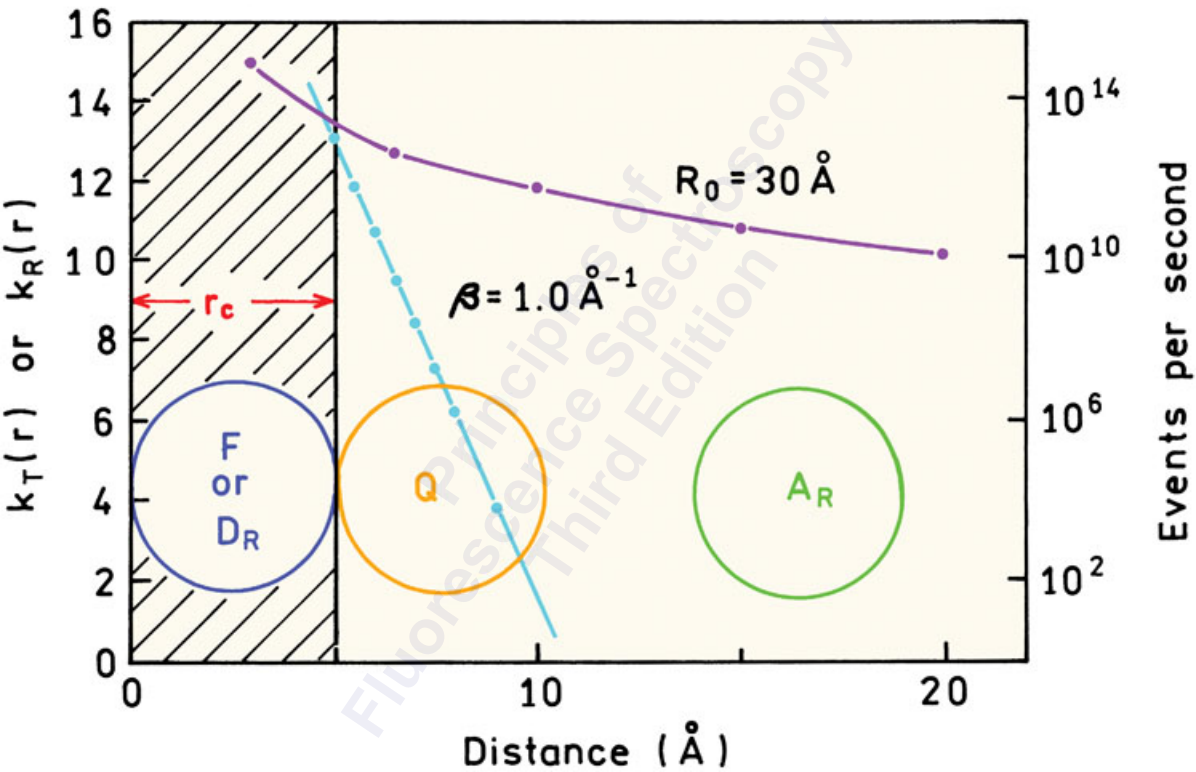


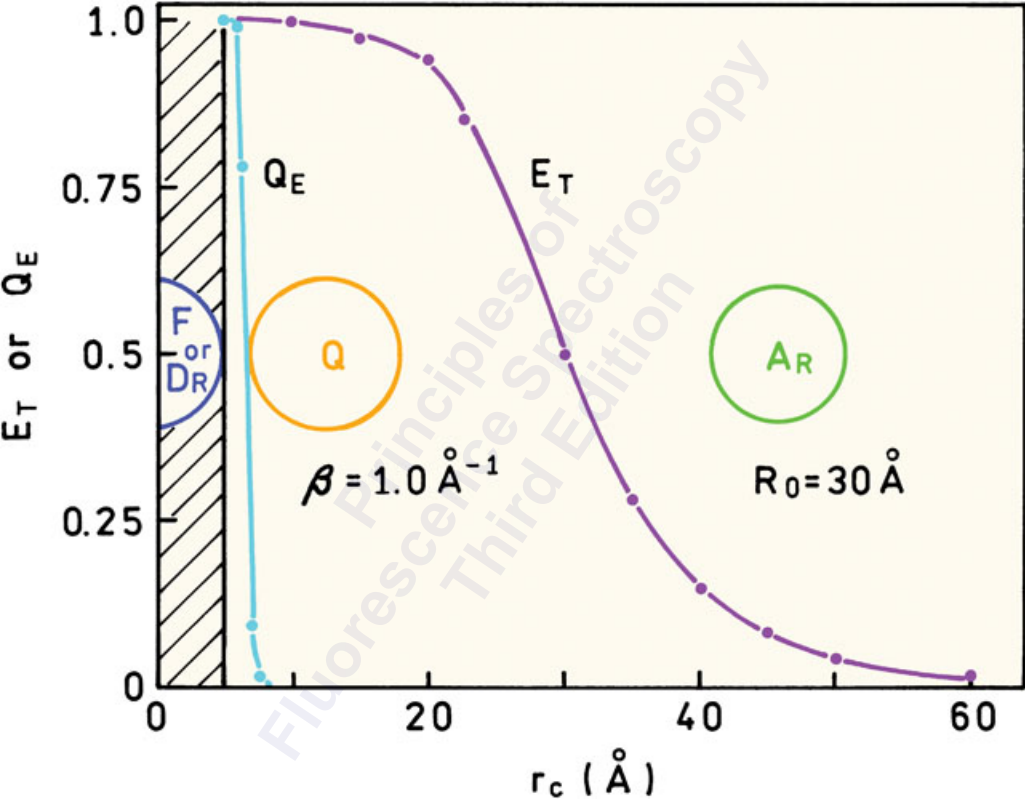
$F^*$

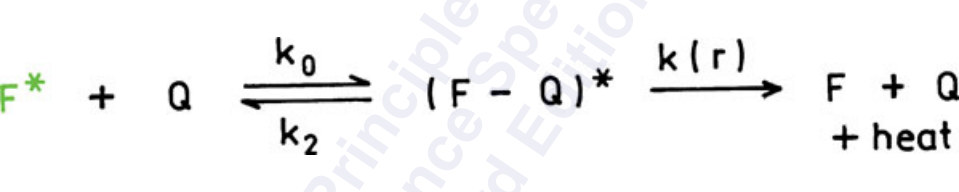
Q

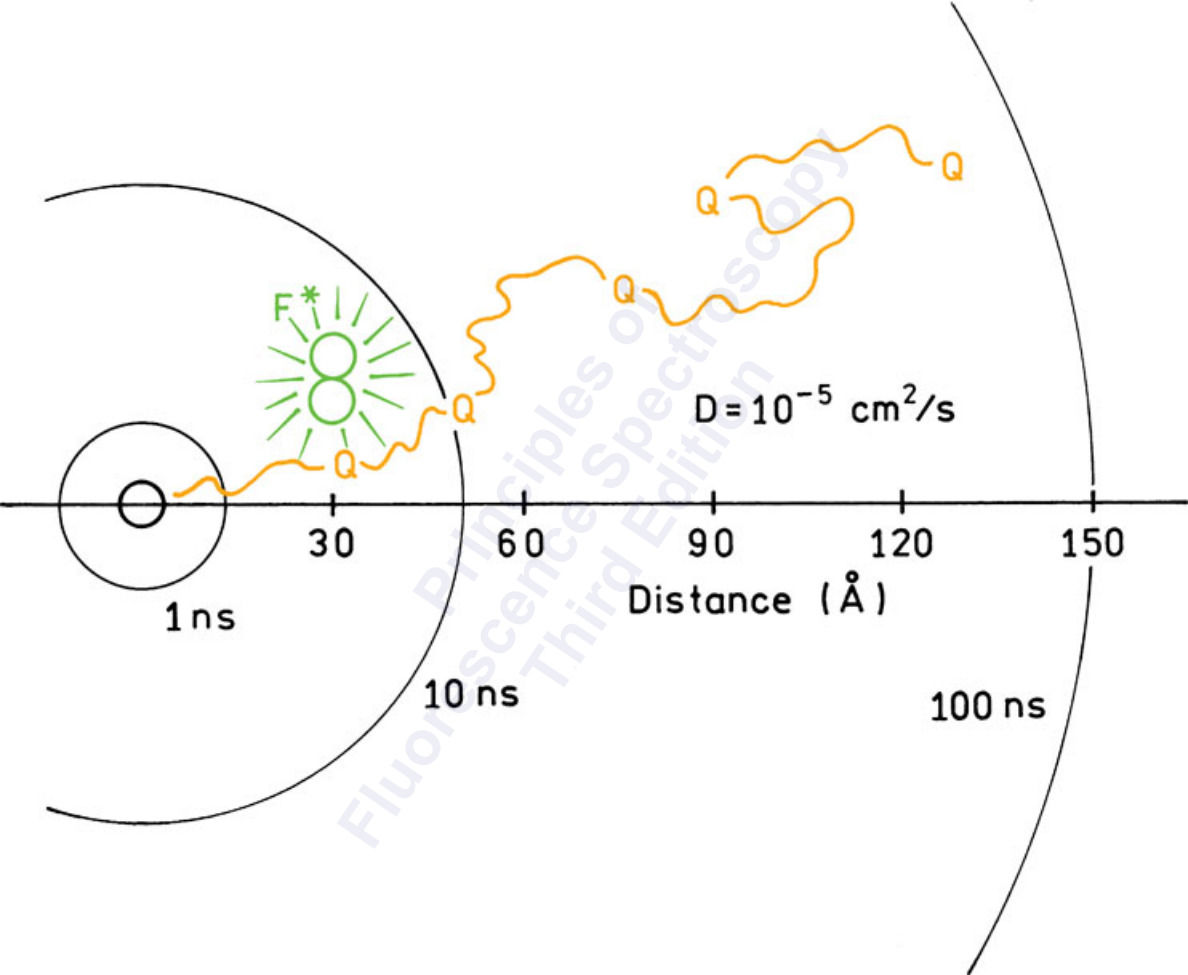
F

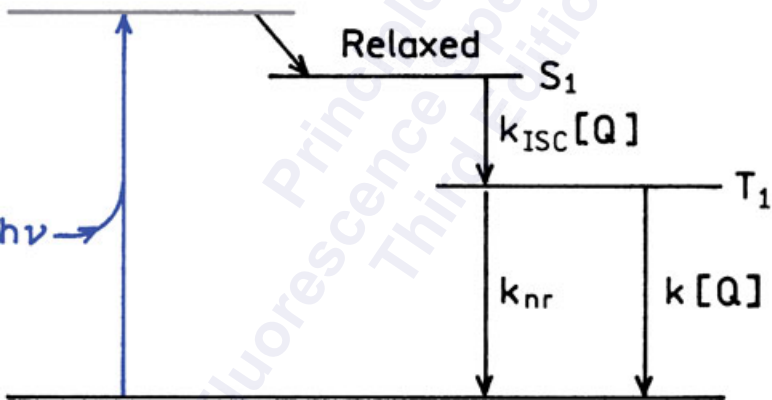
Q

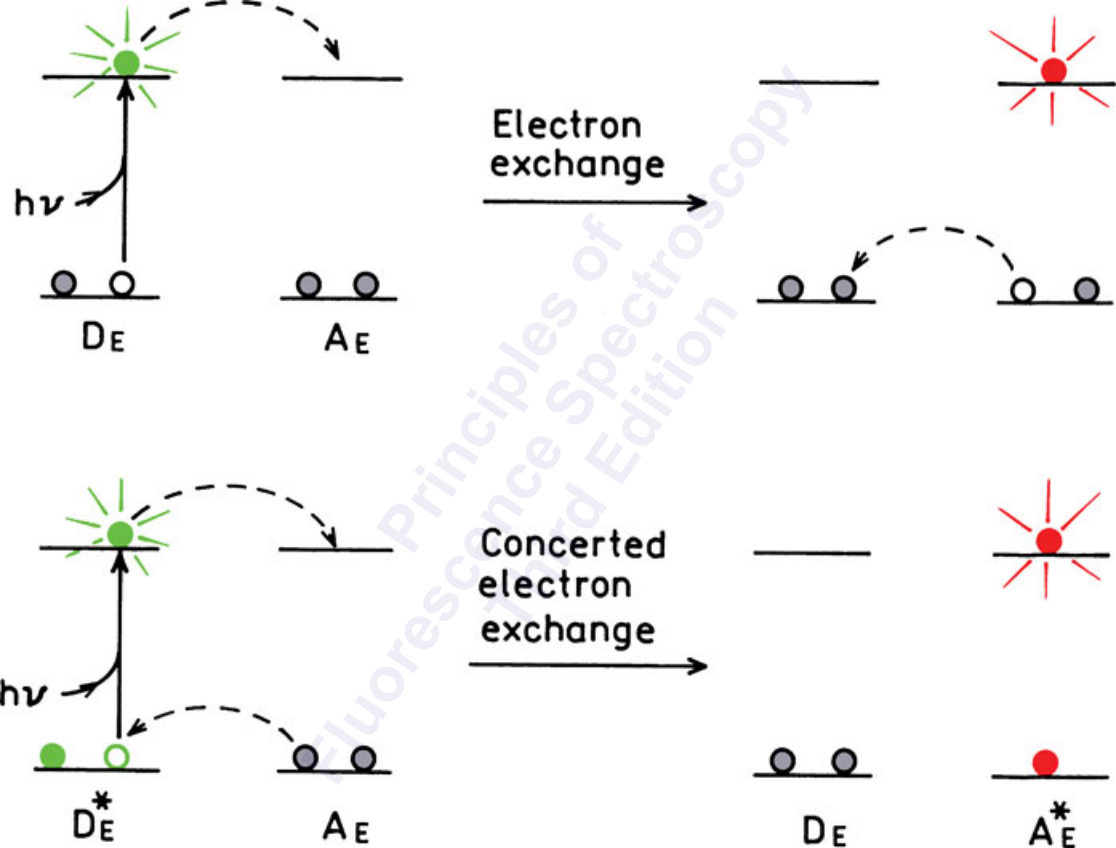


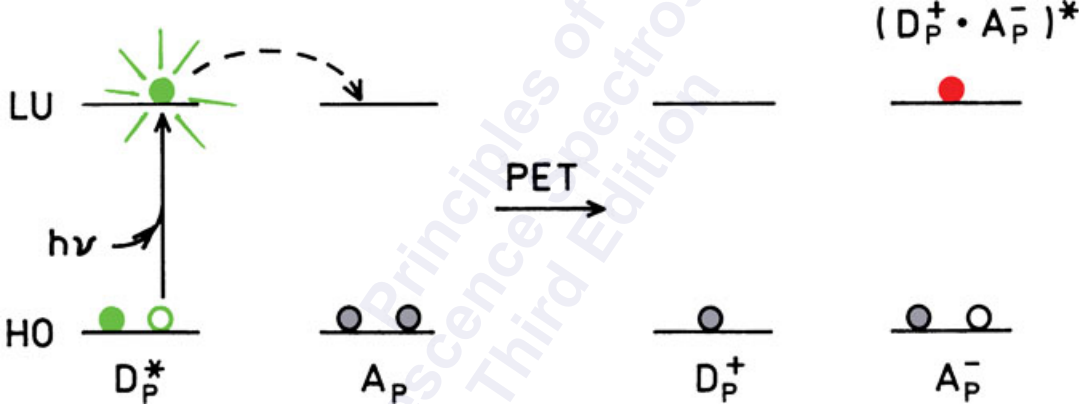




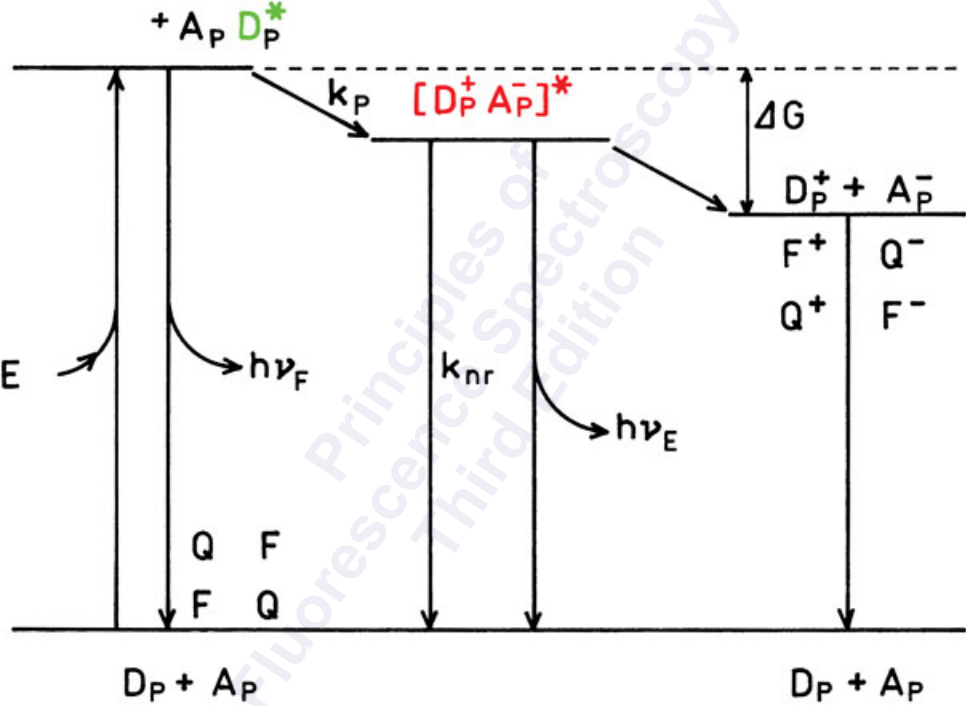


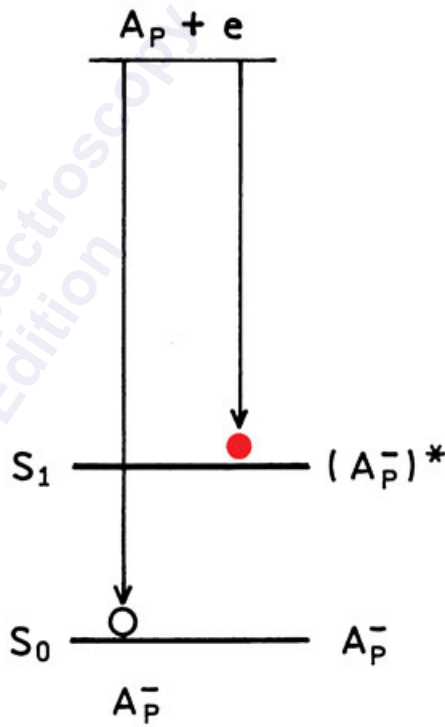
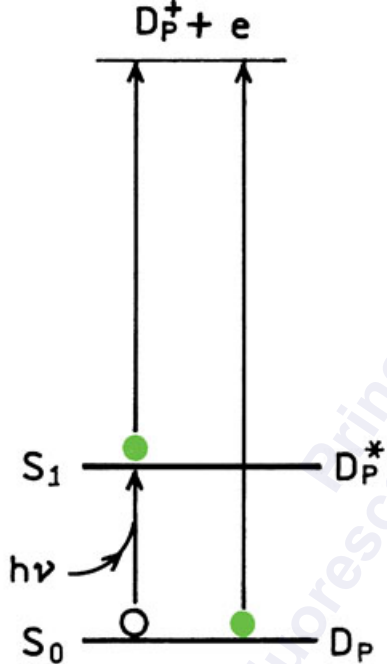


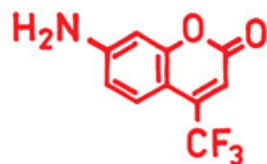




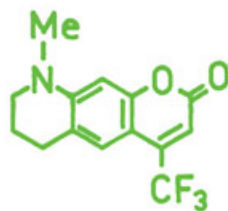




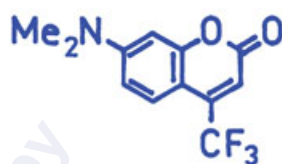




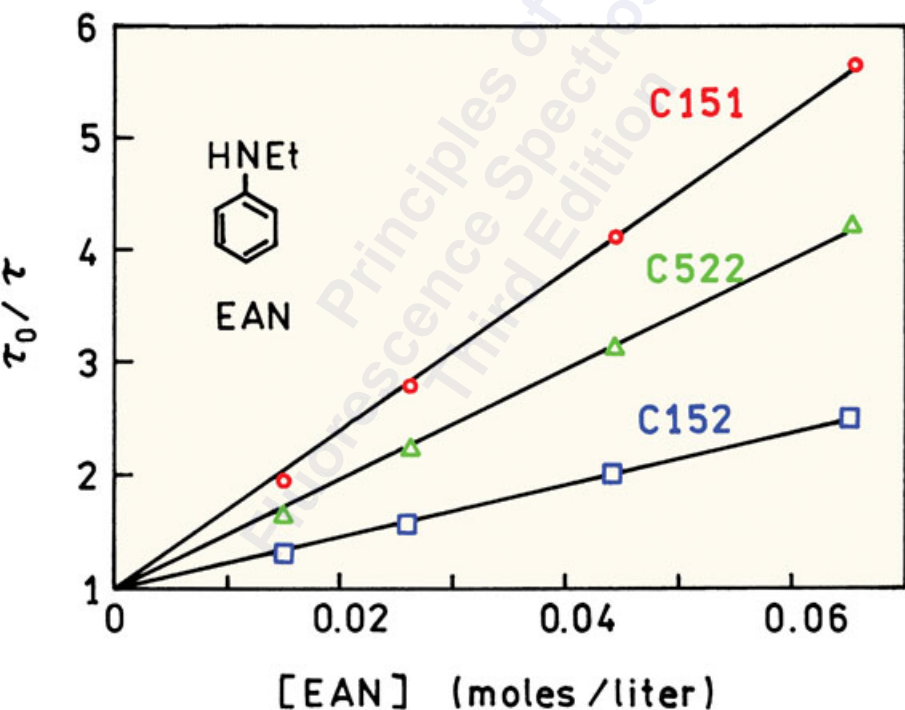
C 151

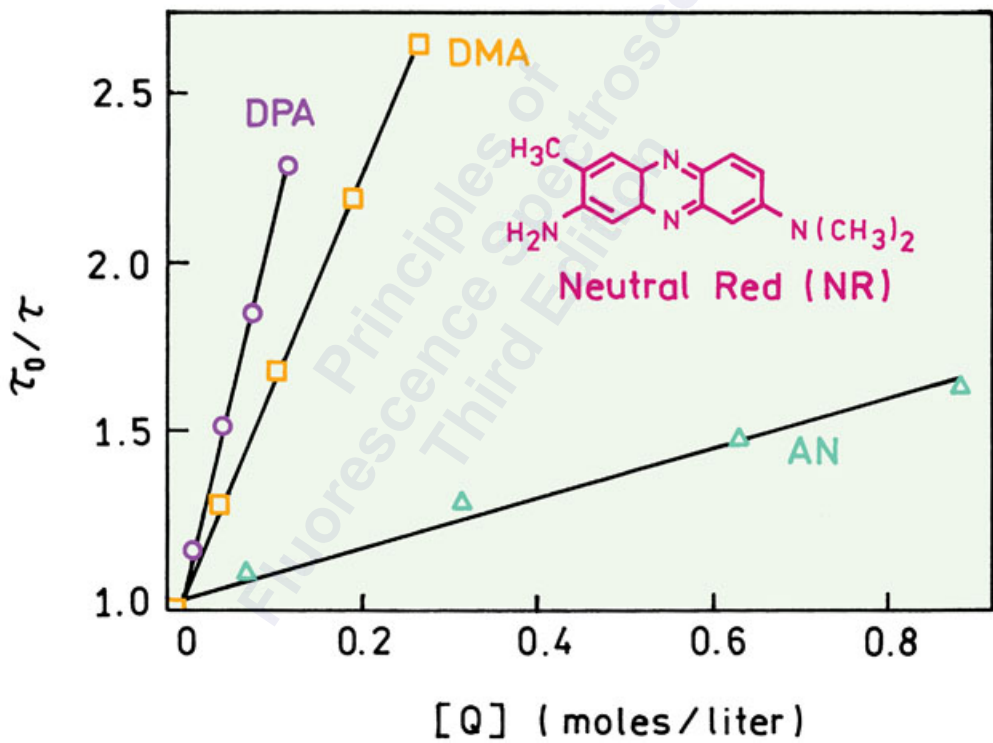
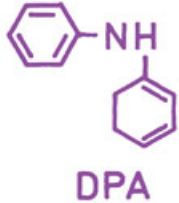


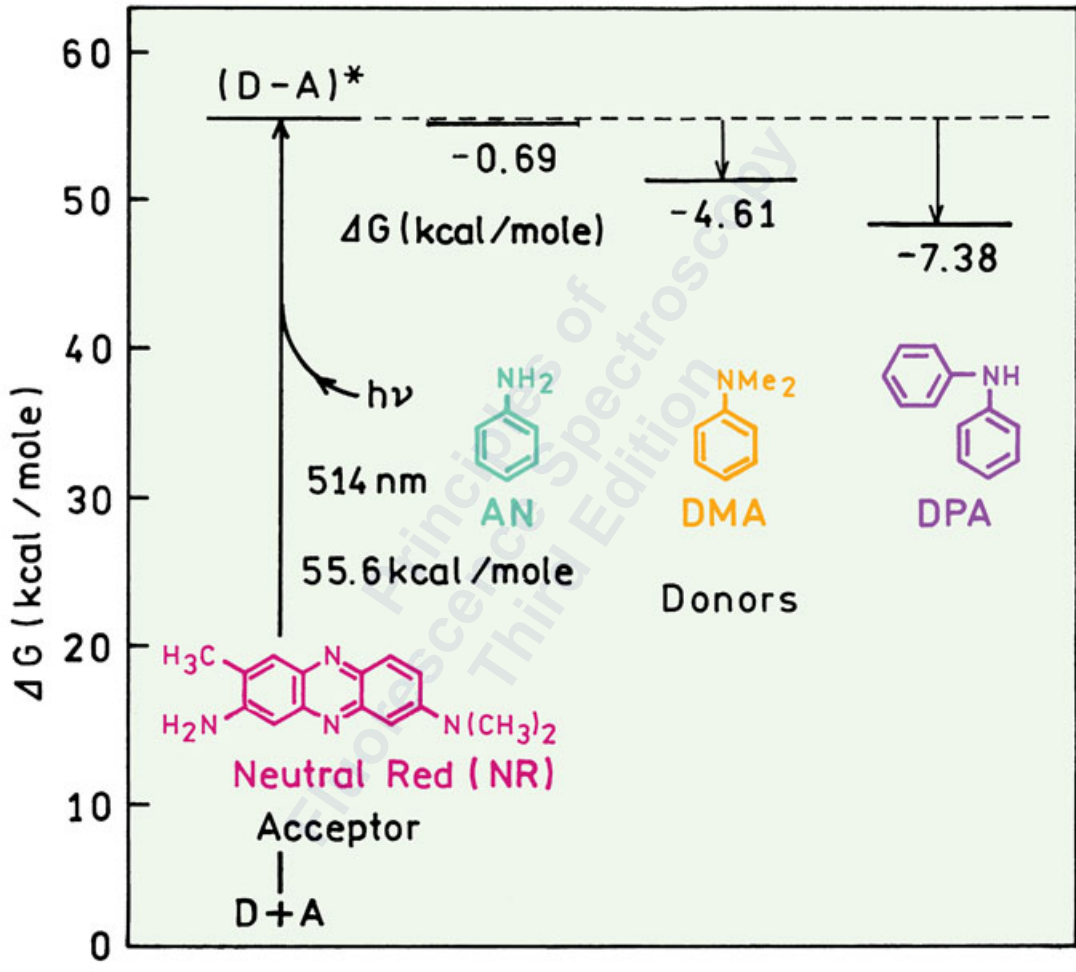
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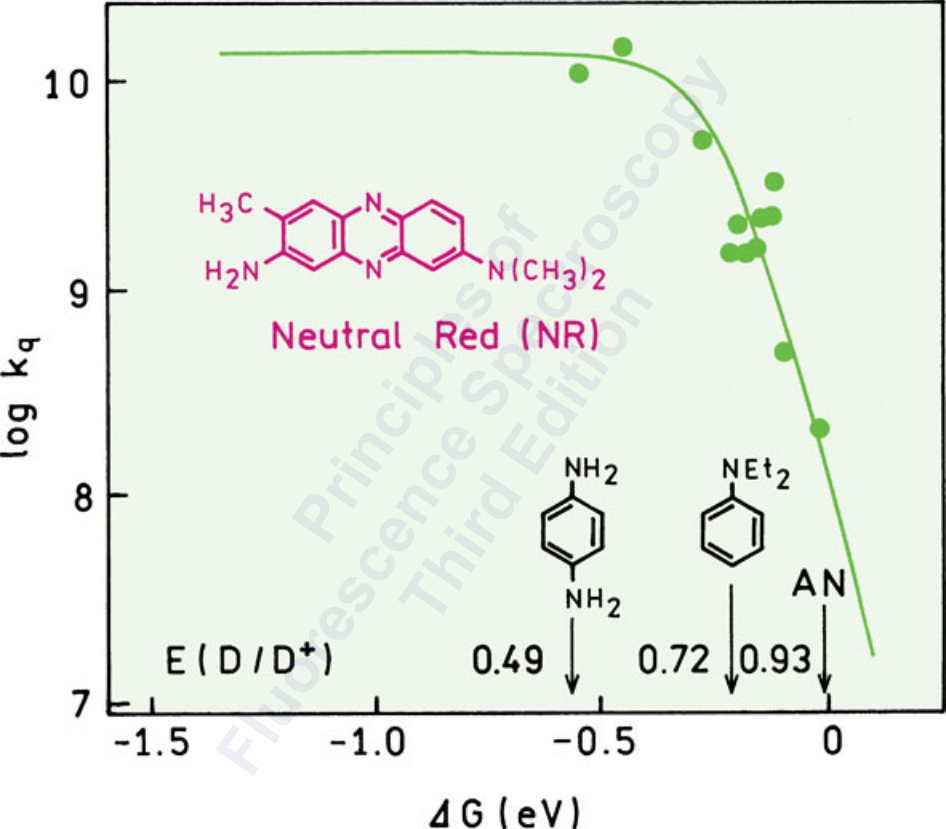


C152

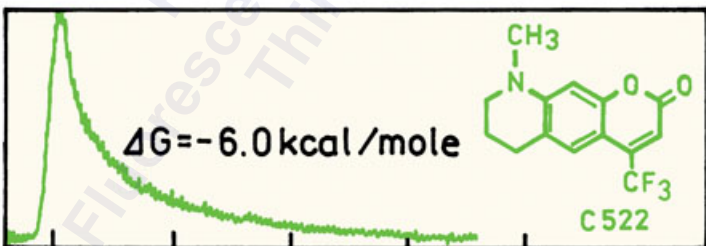
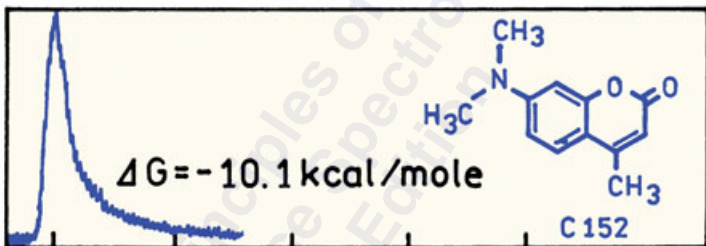
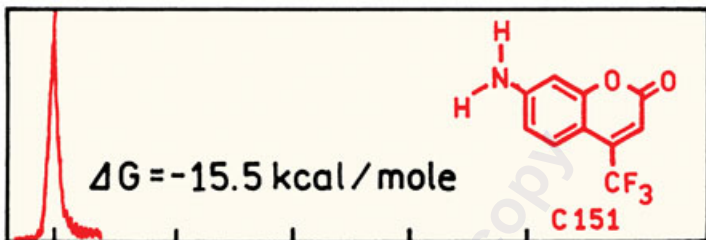




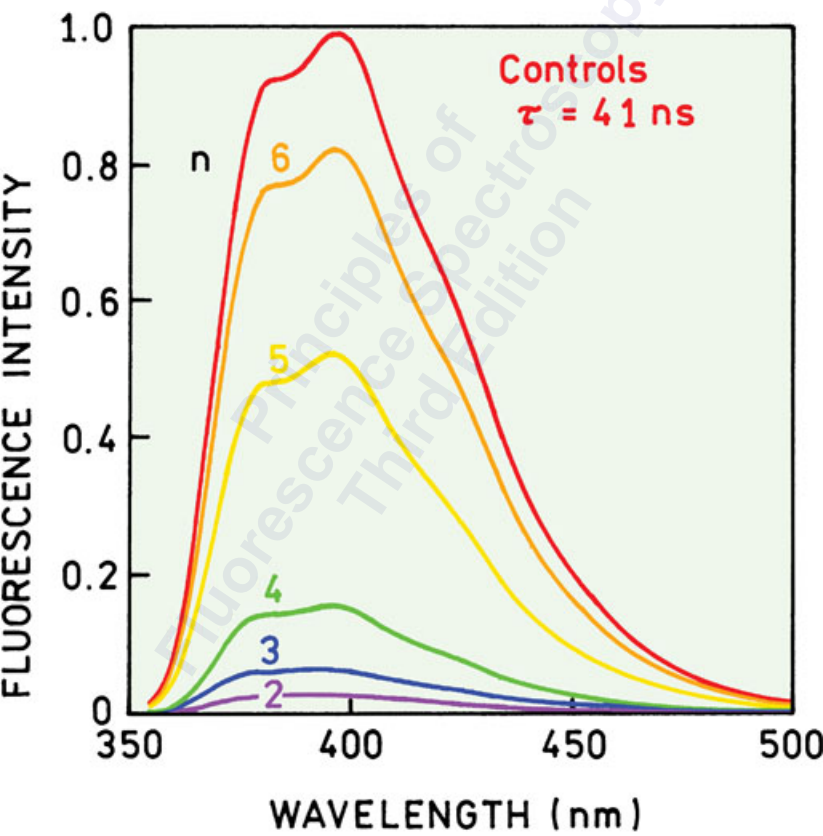
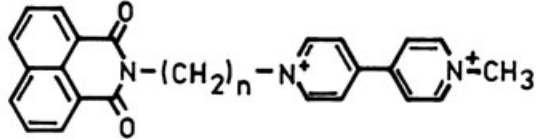




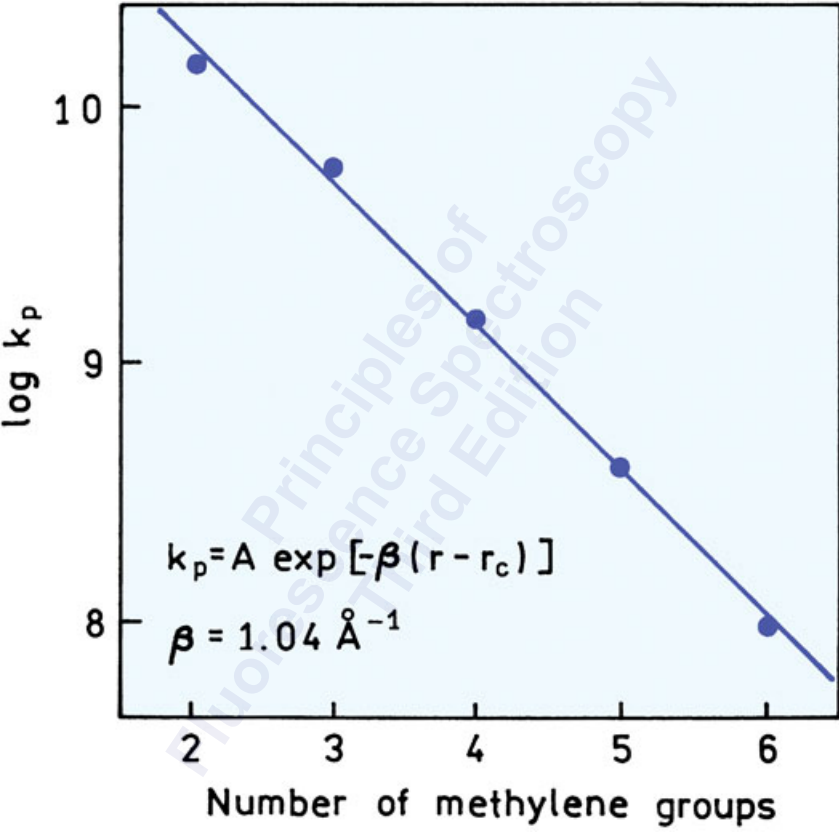
$I(t)$

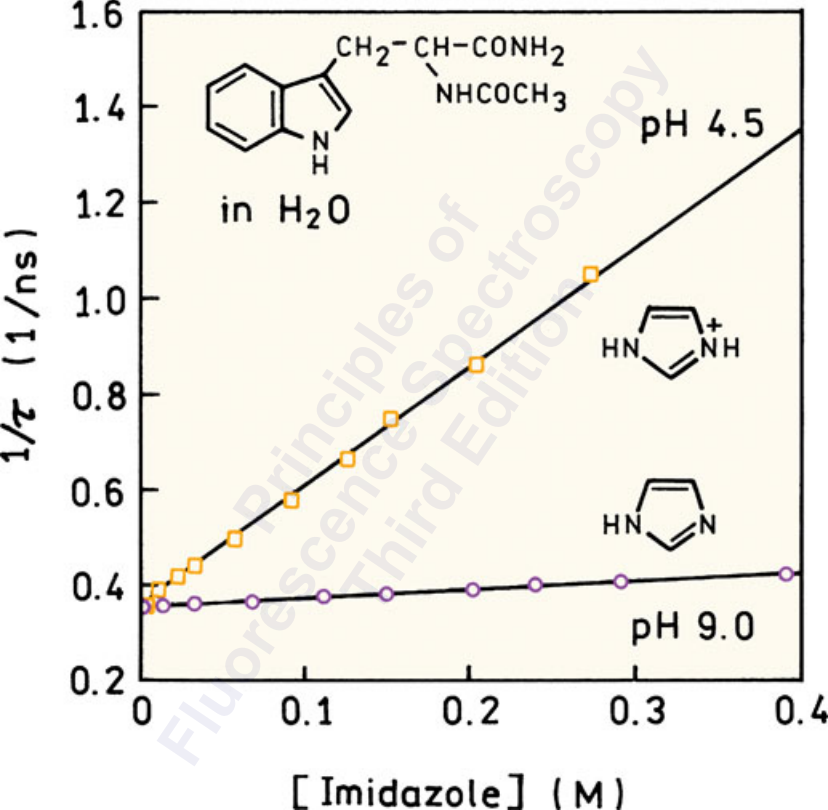


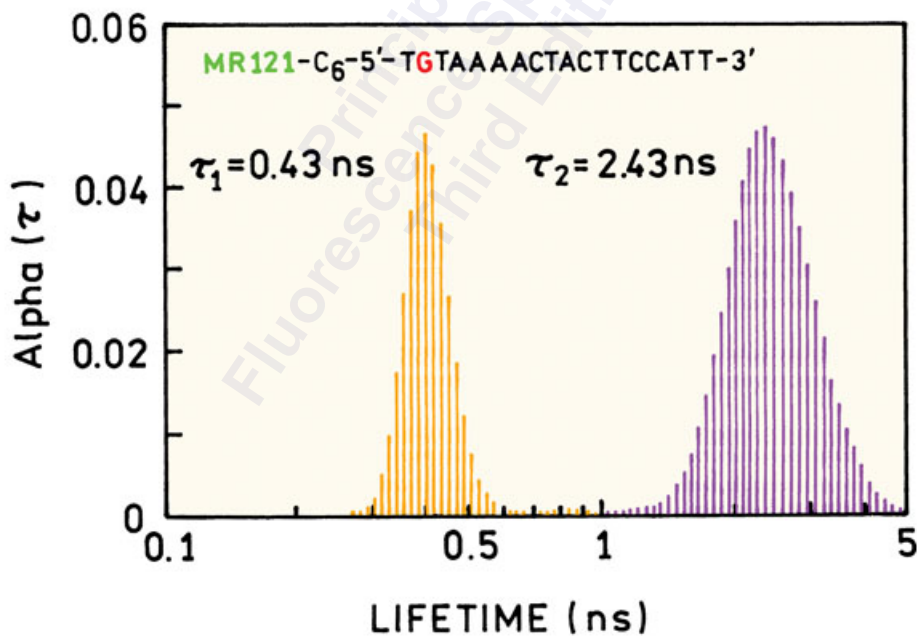
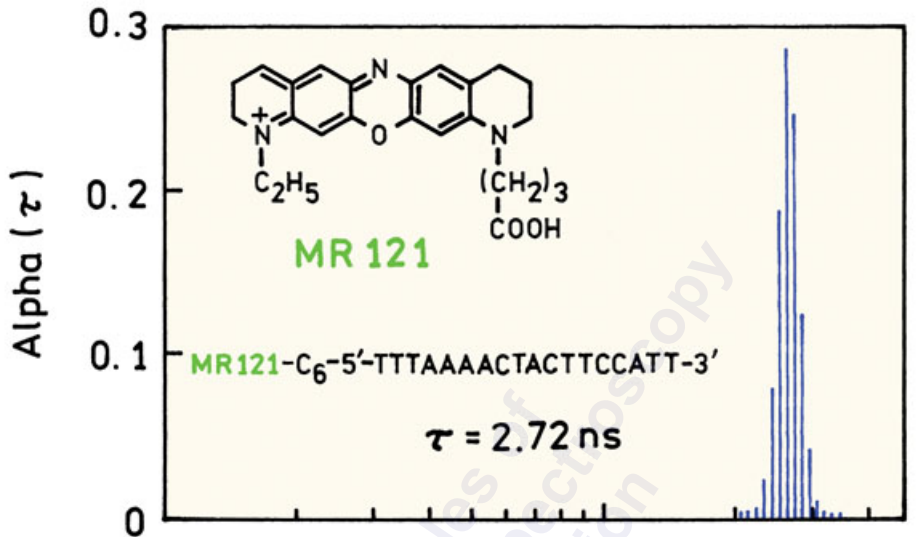
TIME (ps)

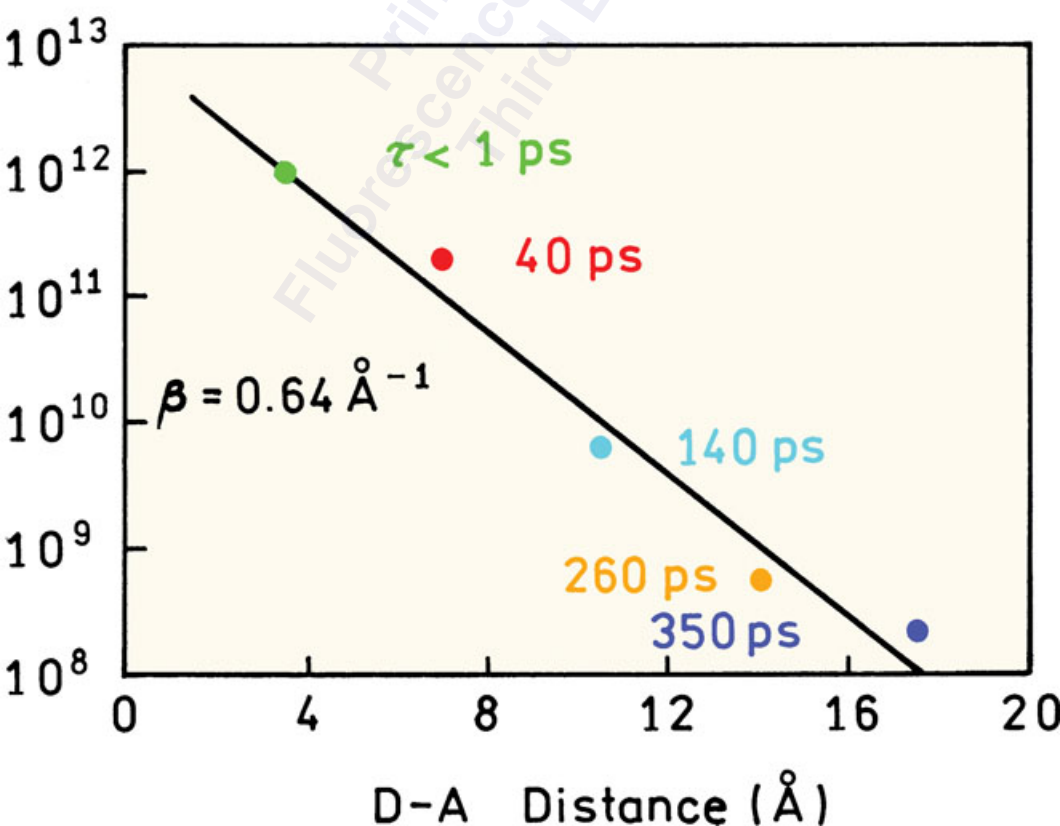
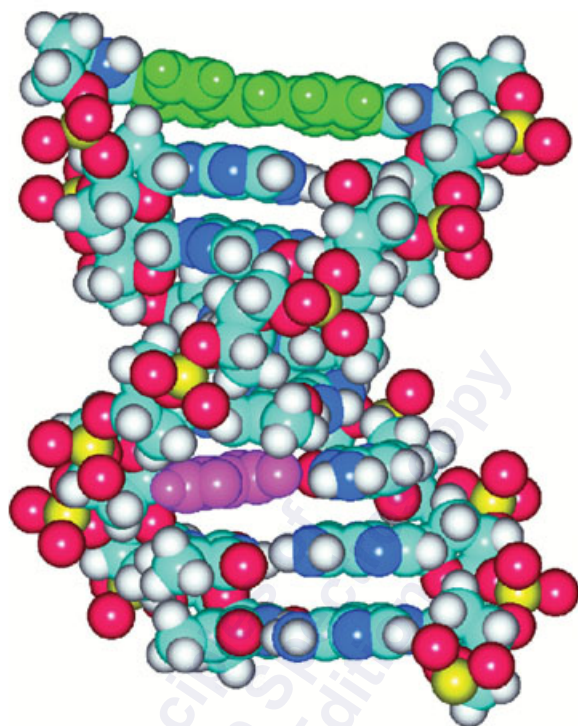
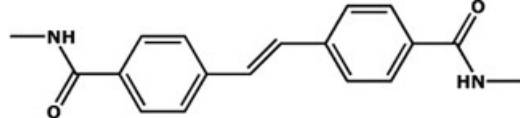


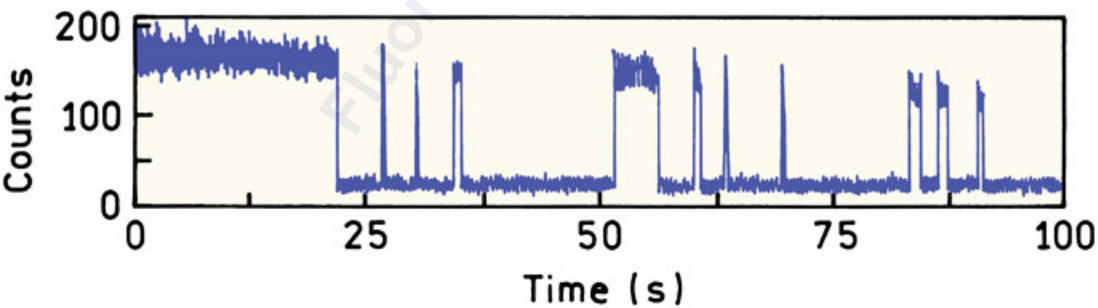
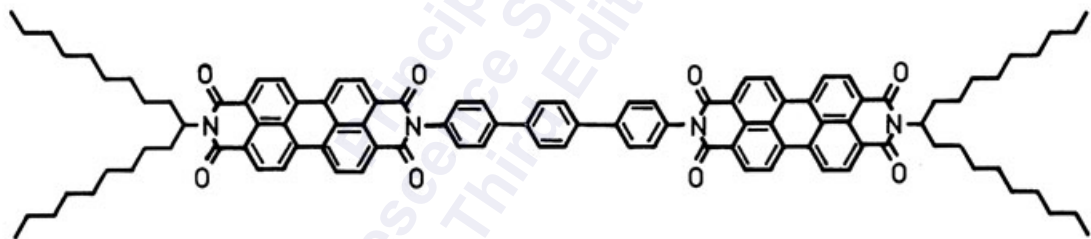
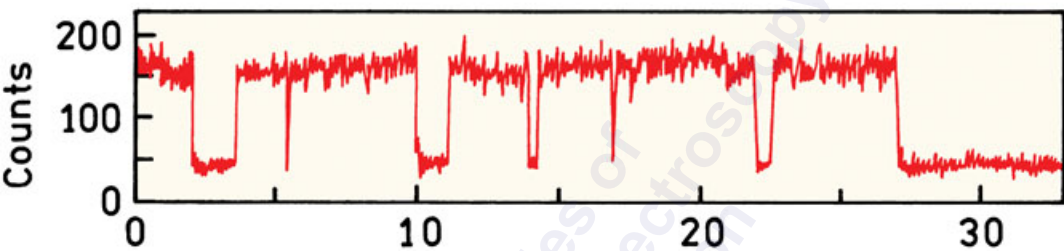
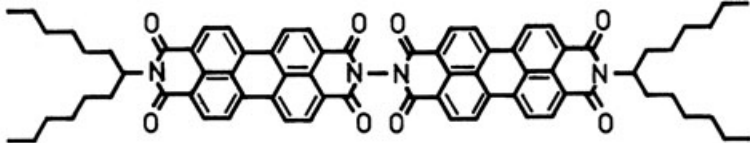






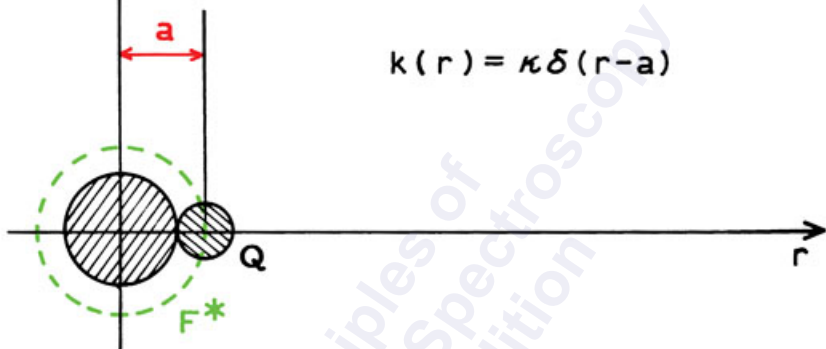






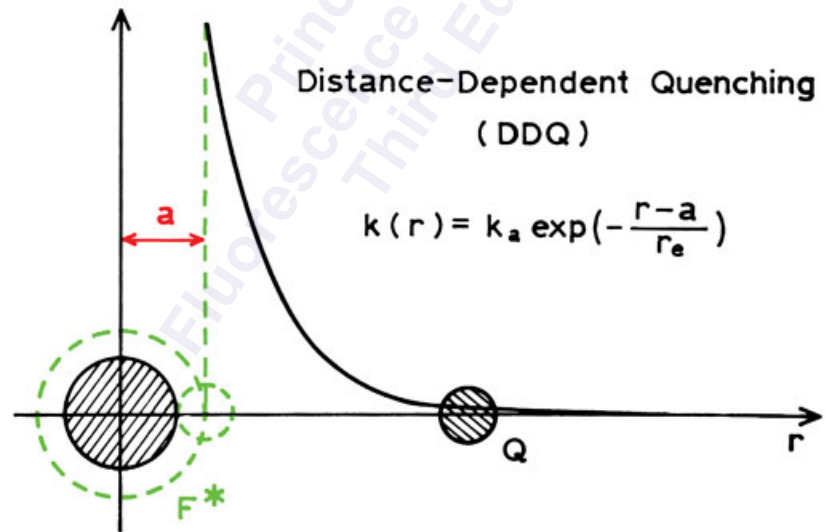
## Radiation Boundary Condition (RBC)

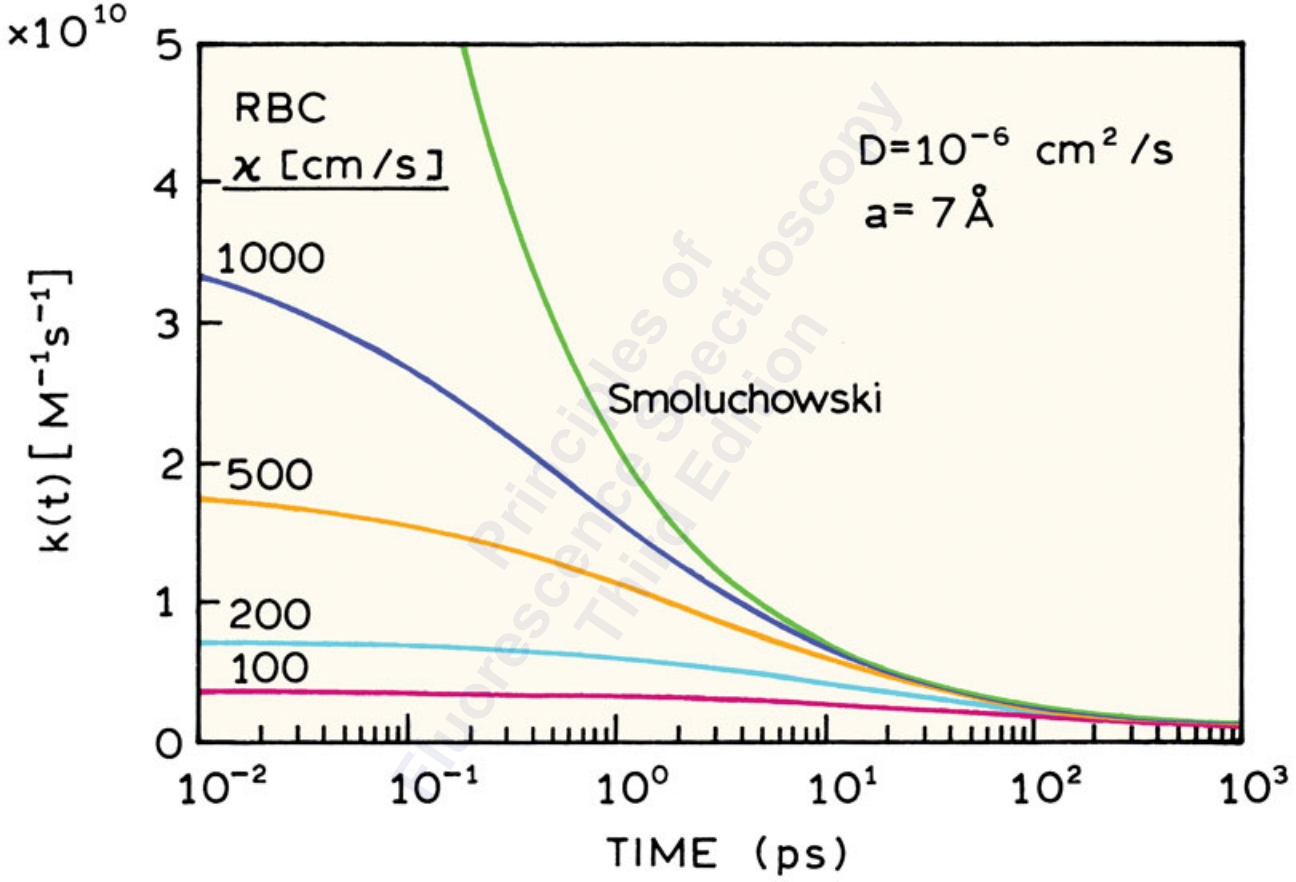
$$k(r) = \kappa \delta(r-a)$$

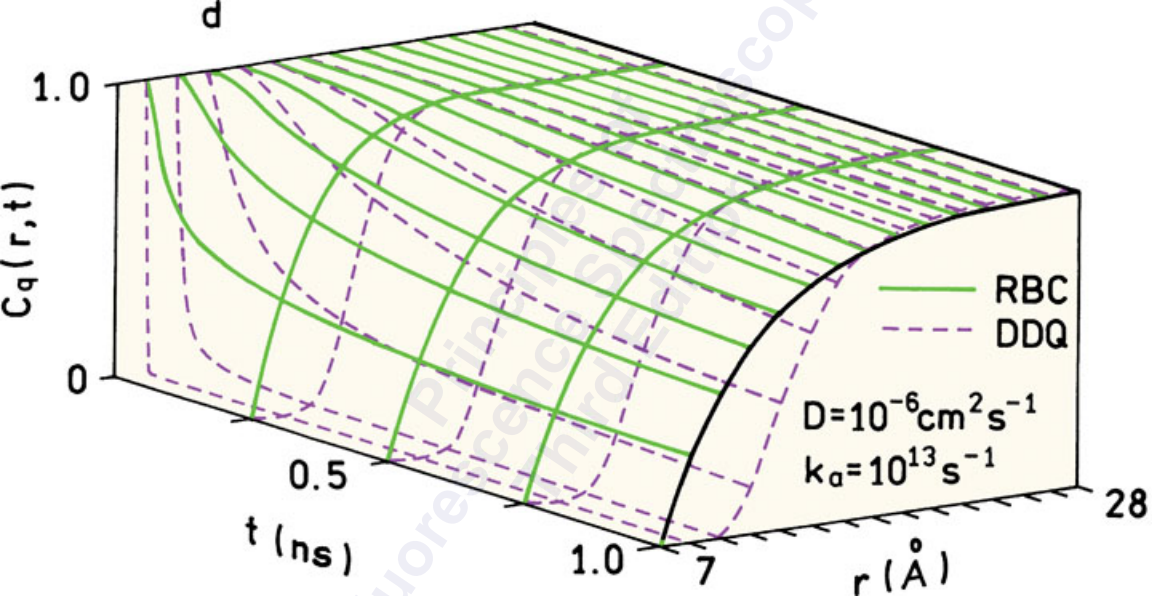


## Distance-Dependent Quenching (DDQ)

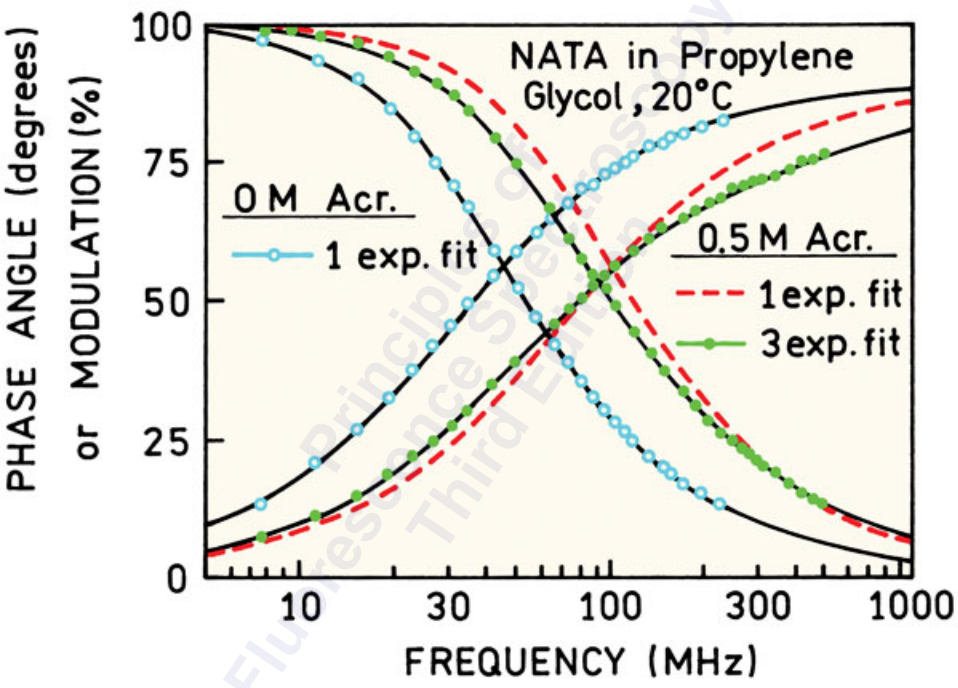
$$k(r) = k_a \exp\left(-\frac{r-a}{r_e}\right)$$

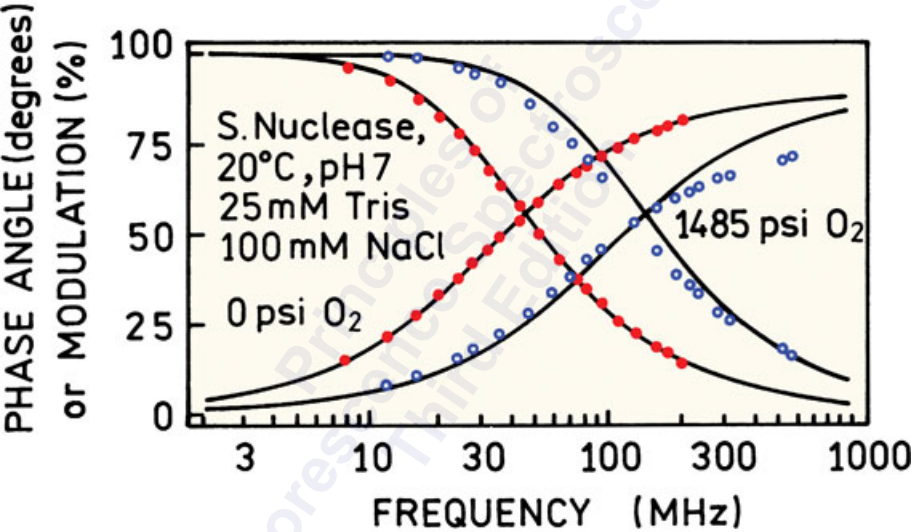


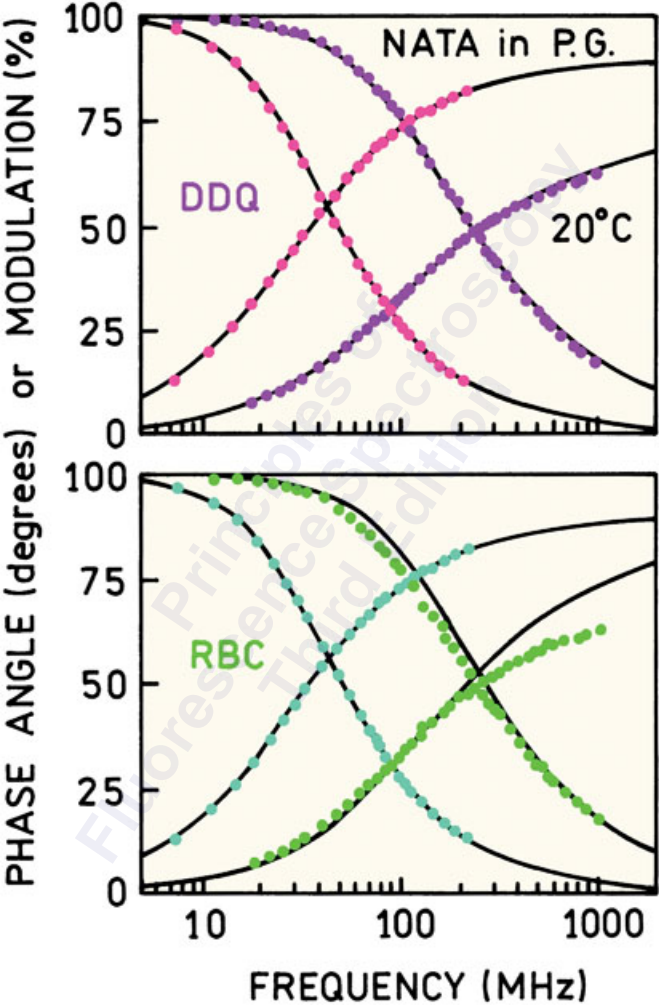


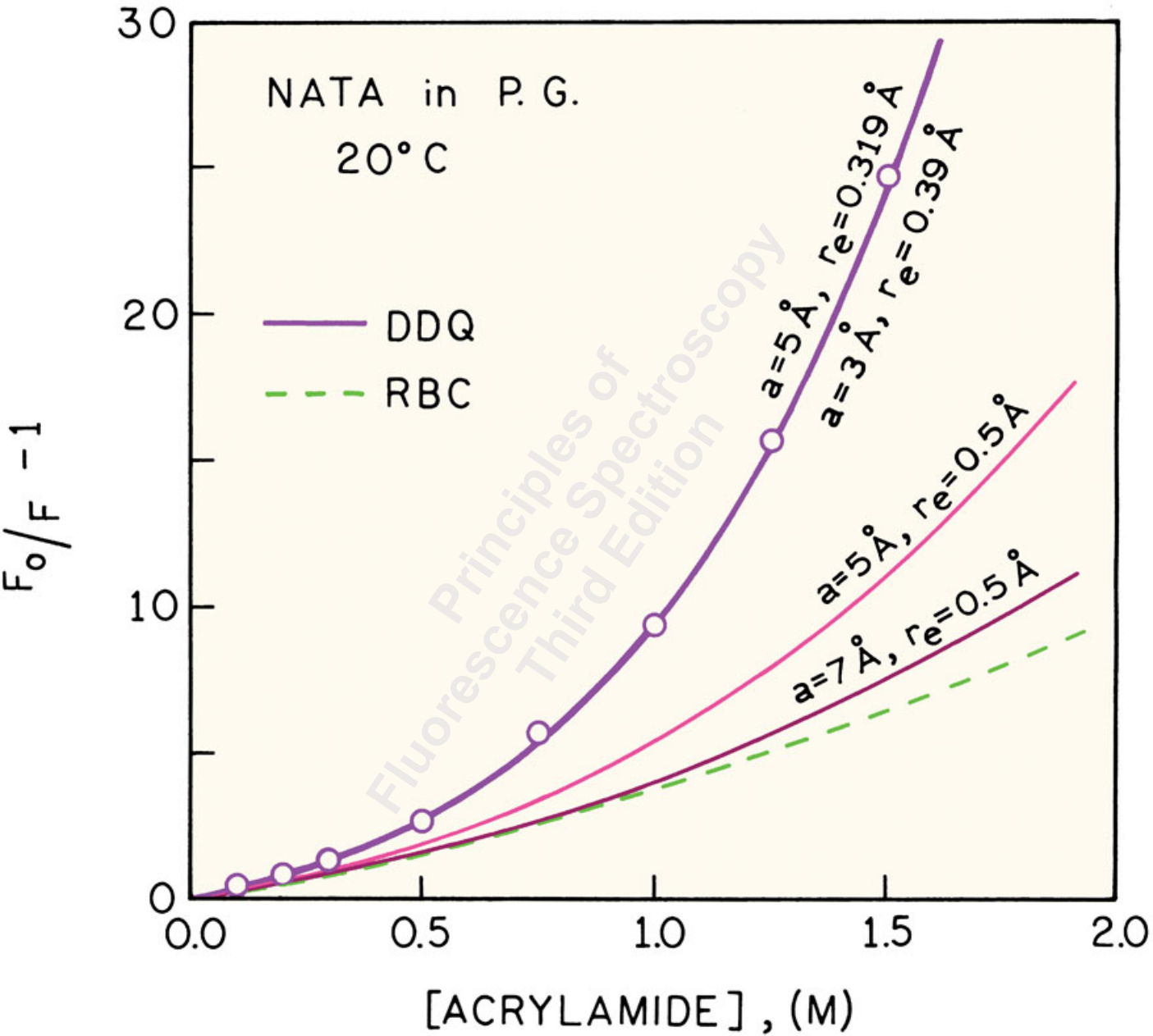


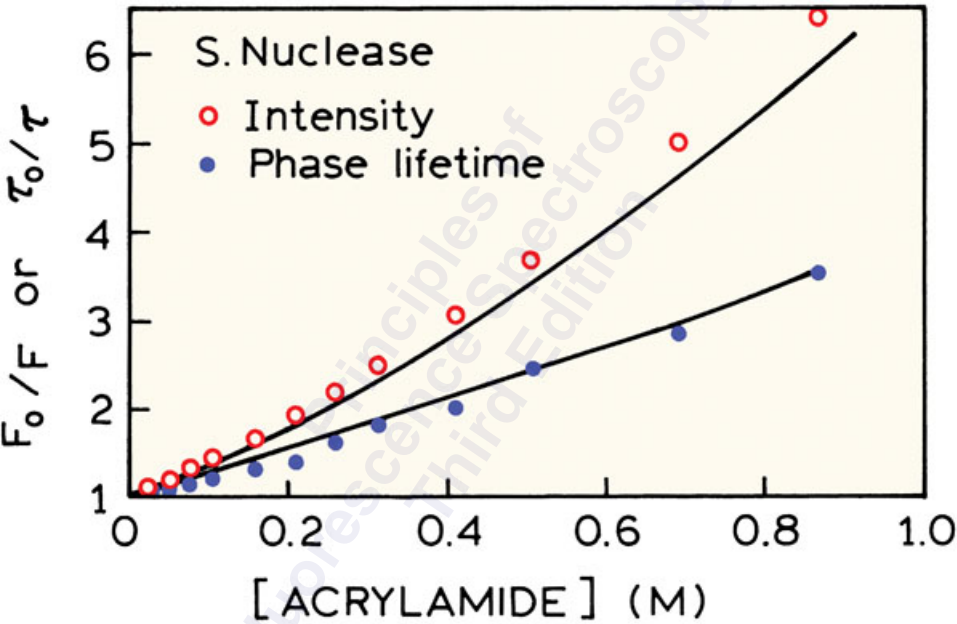


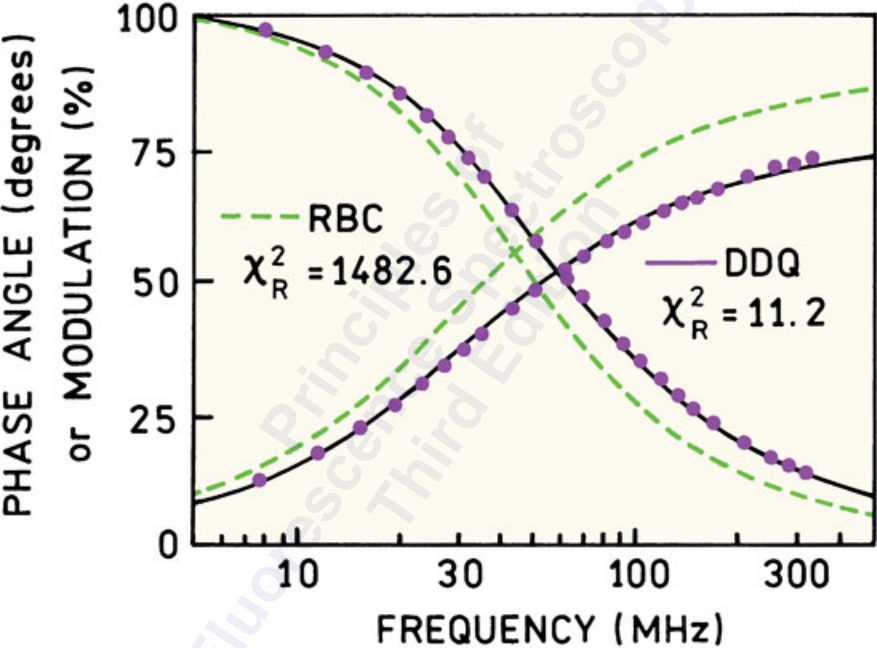


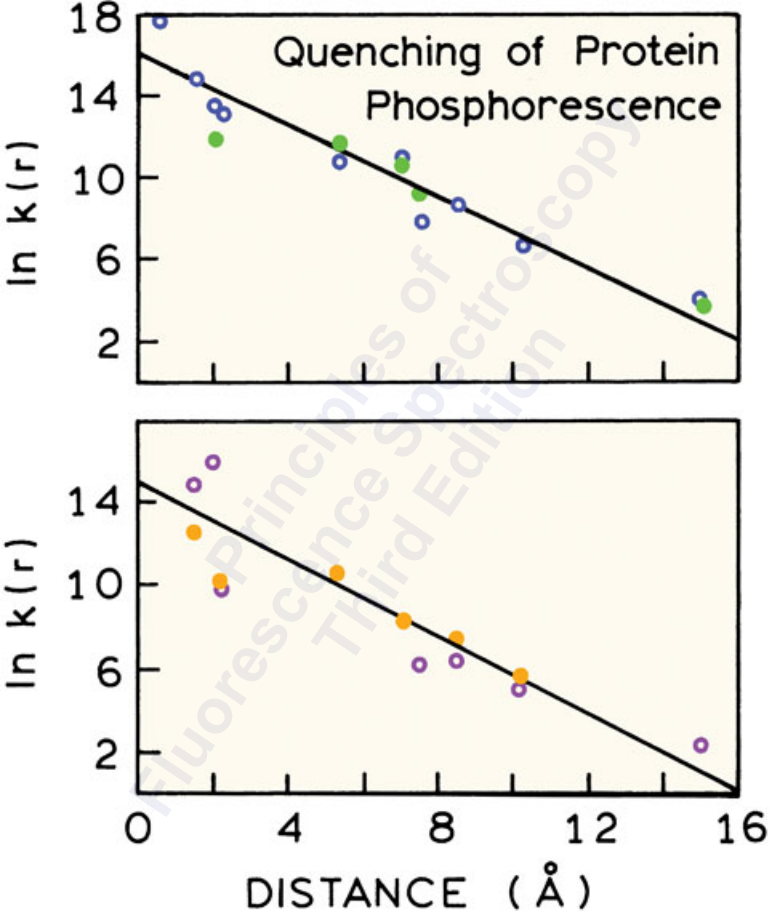


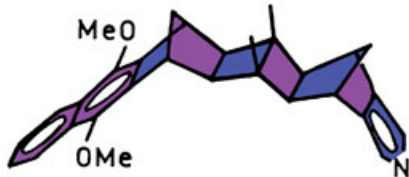




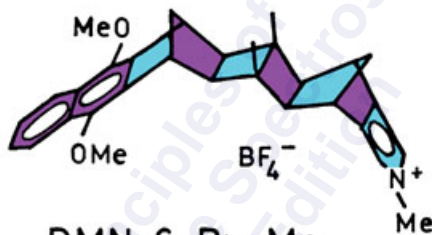




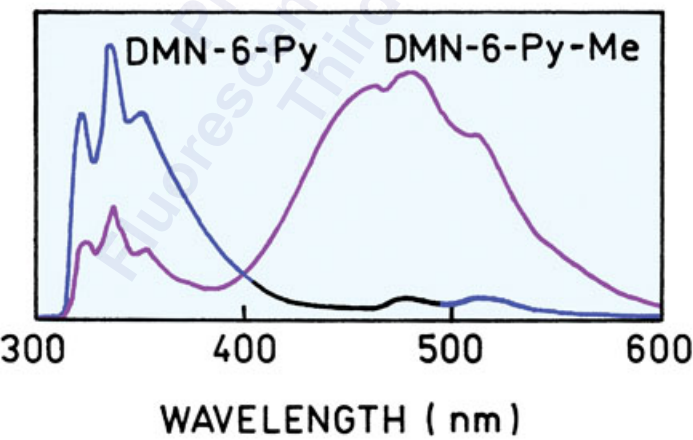




DMN-6 - Py

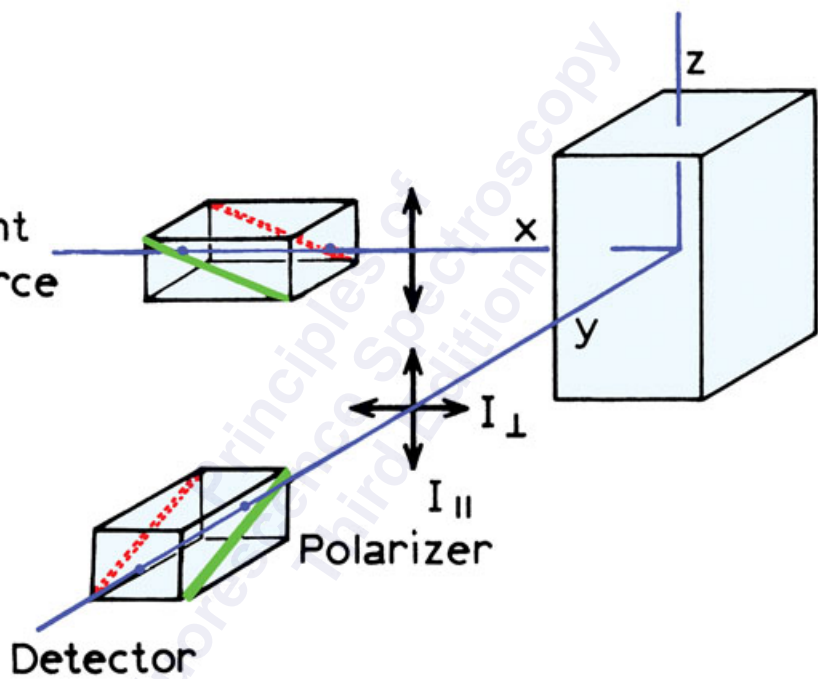


DMN-6-Py-Me



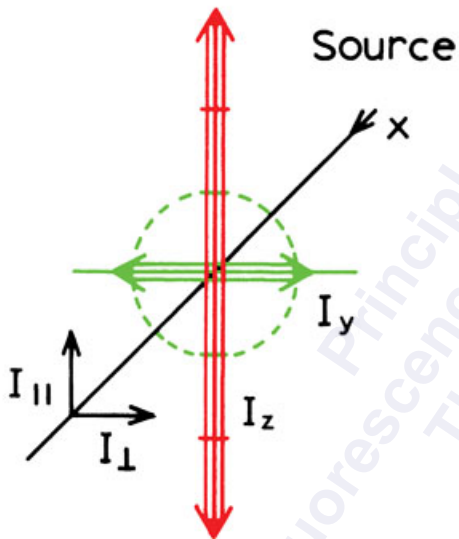


Light  
Source

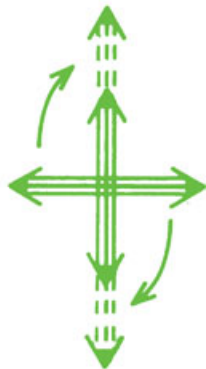


Detector

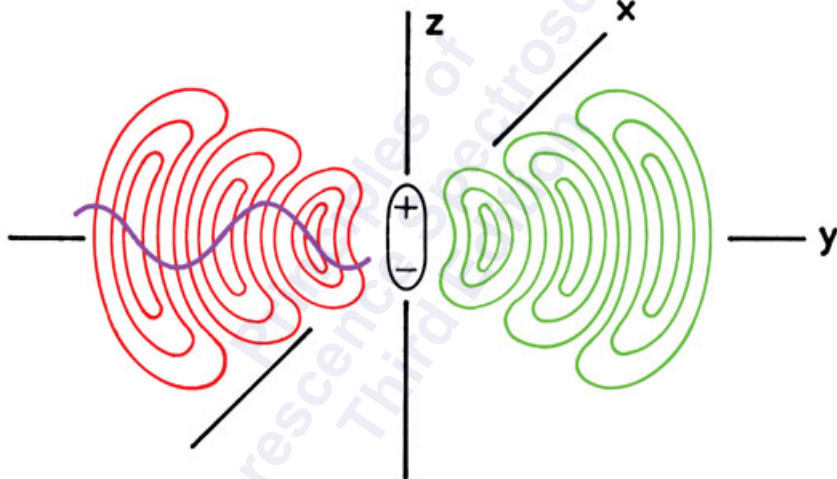
Polarizer

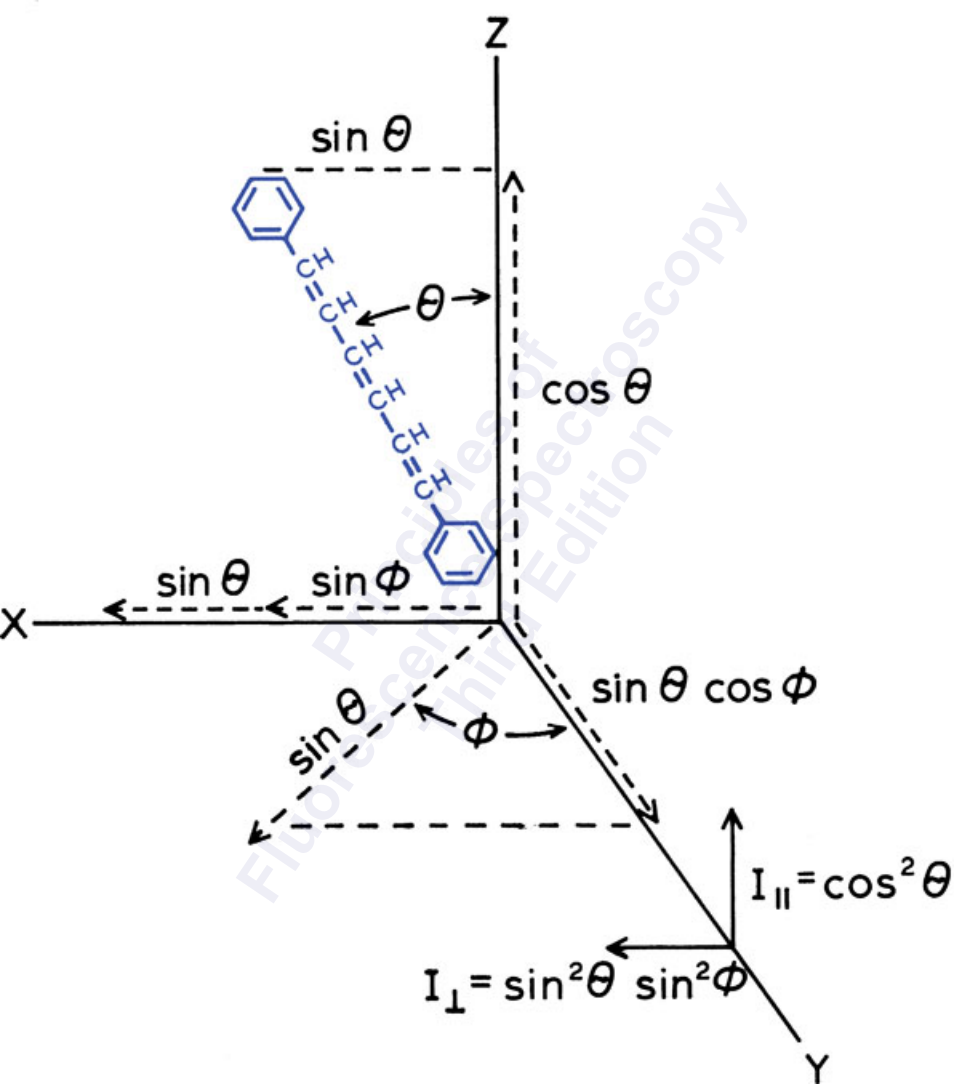


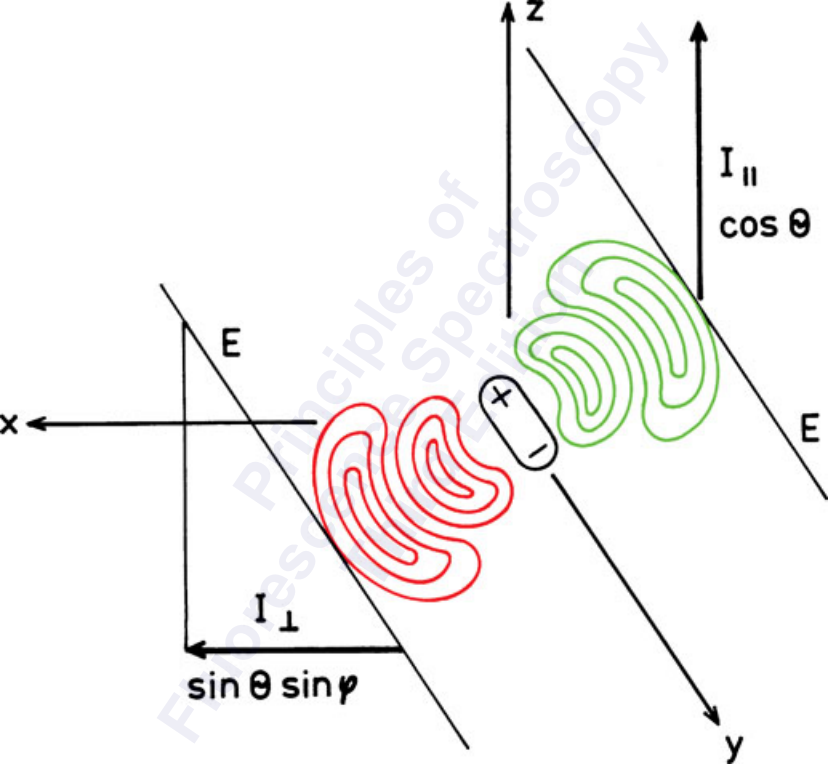
$$p = I_z - I_y$$

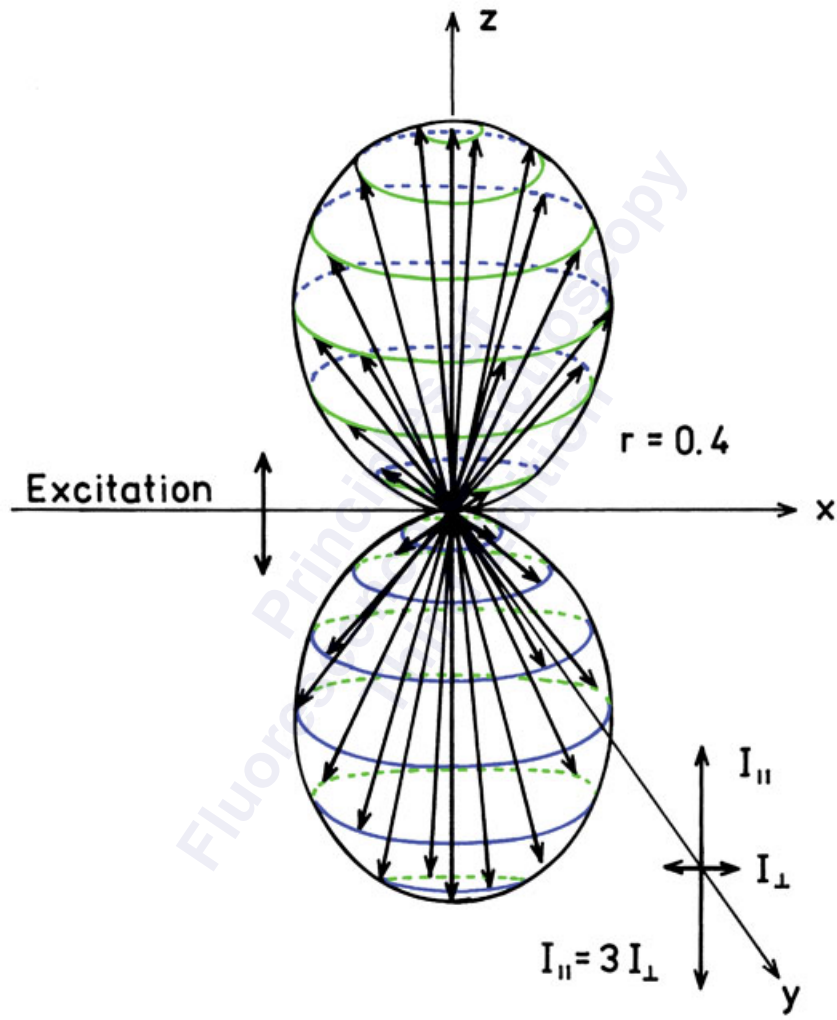


$$n = 2I_y$$

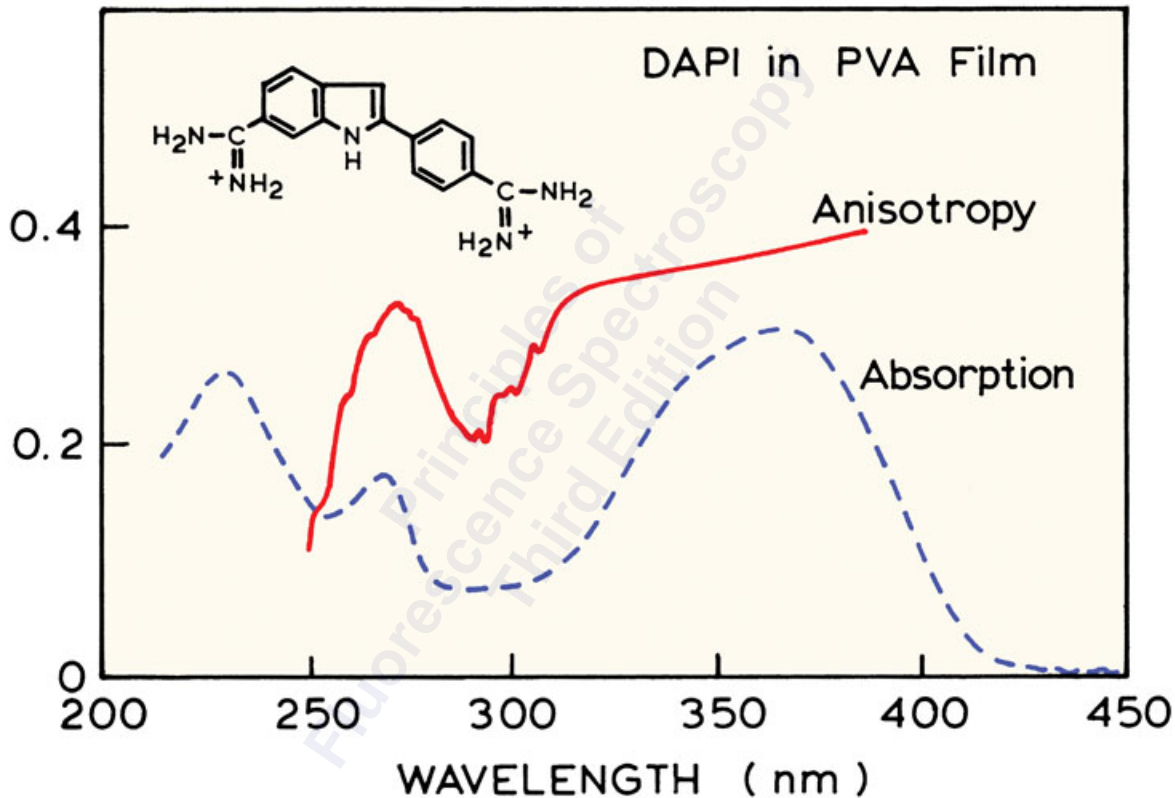


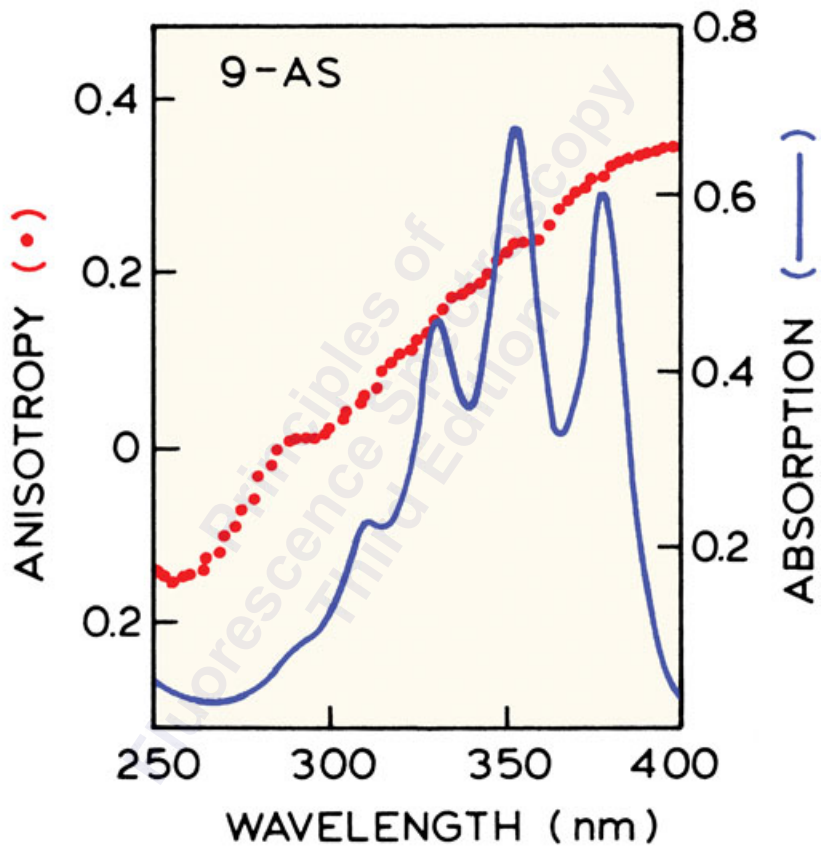




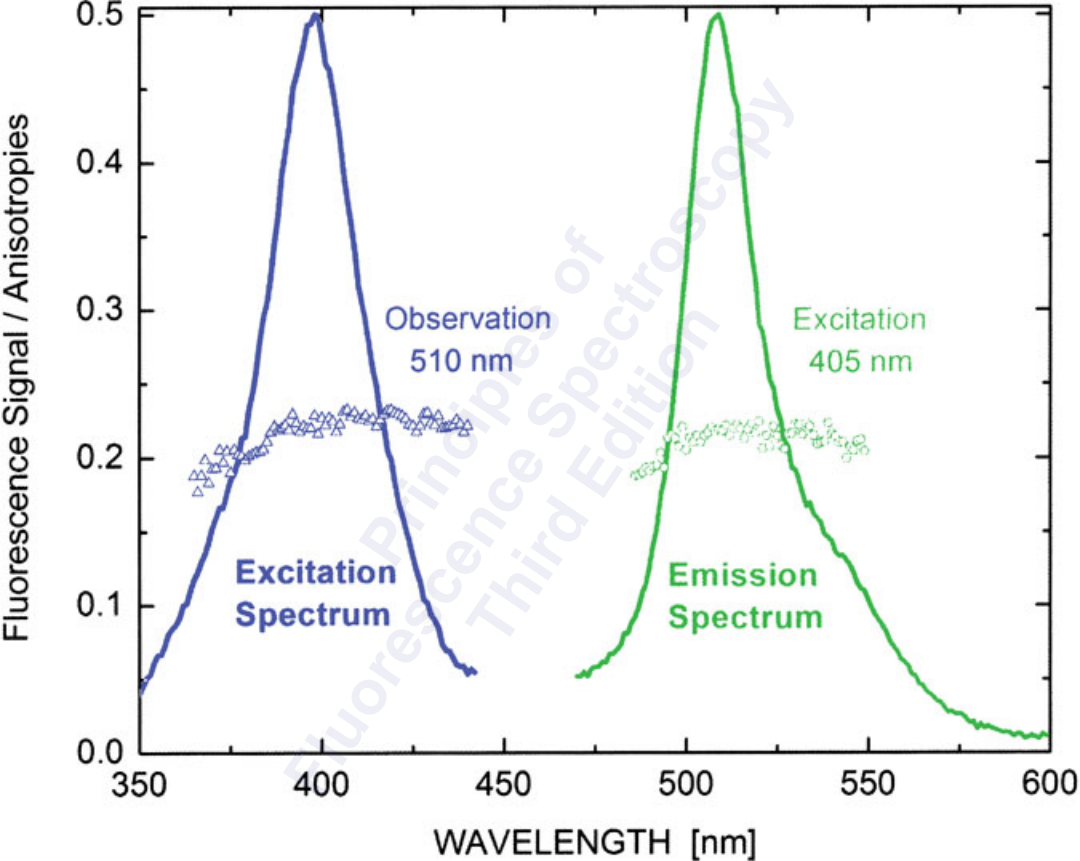


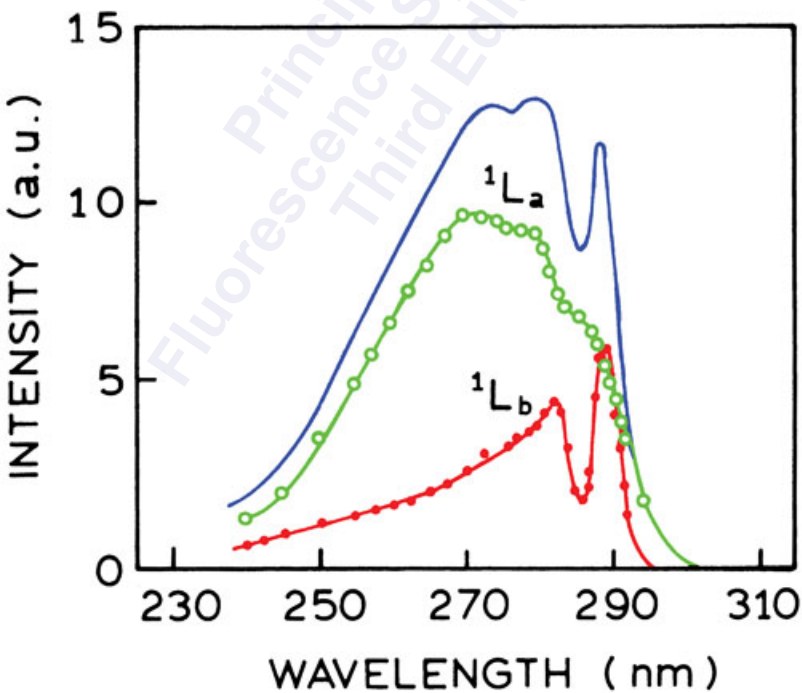
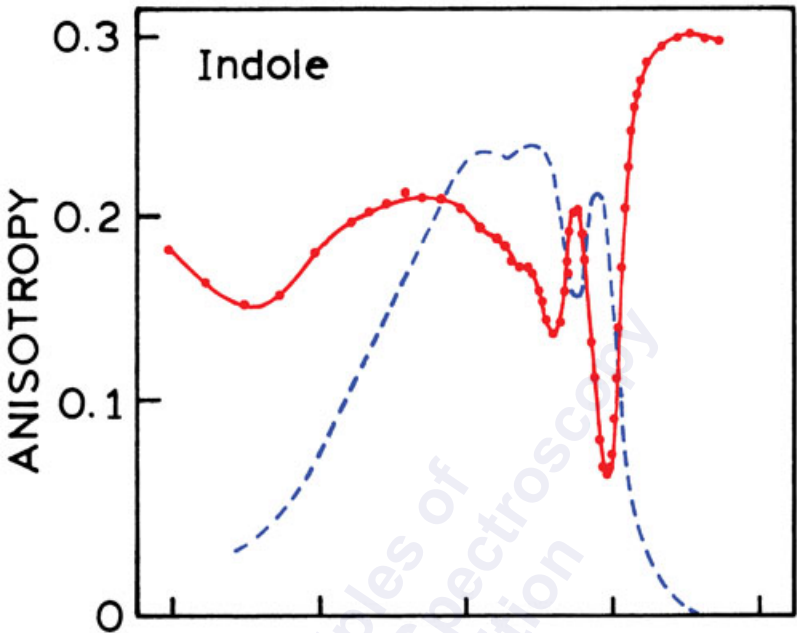
ANISOTROPY

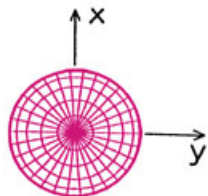
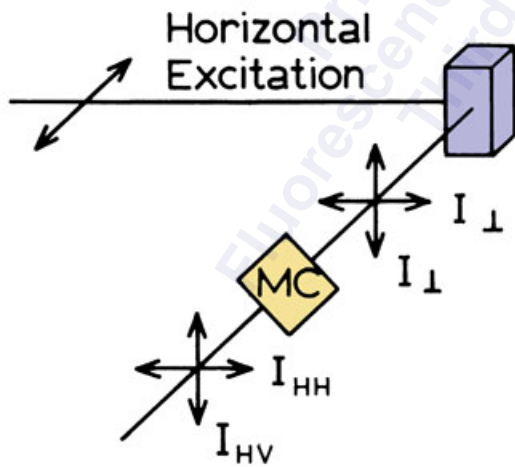
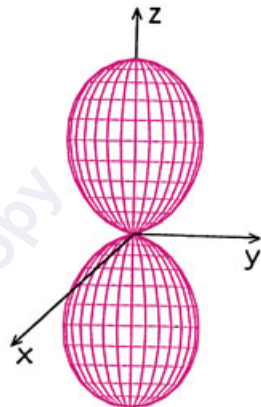
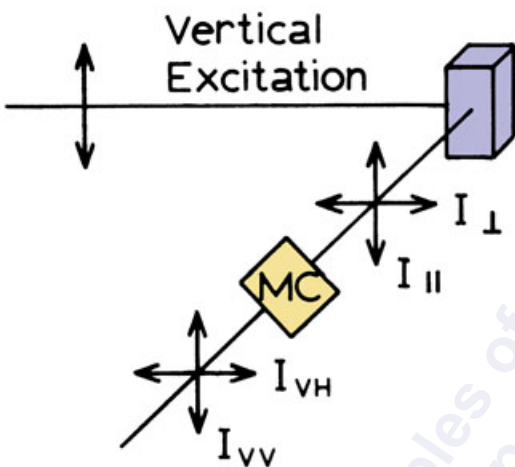




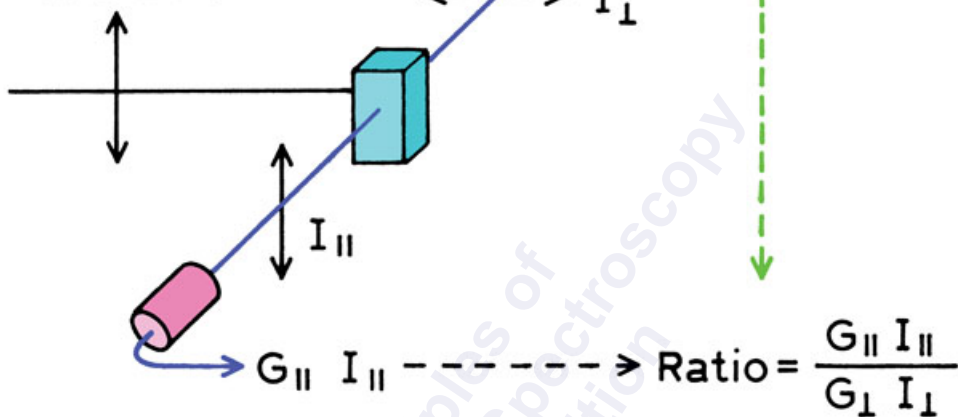




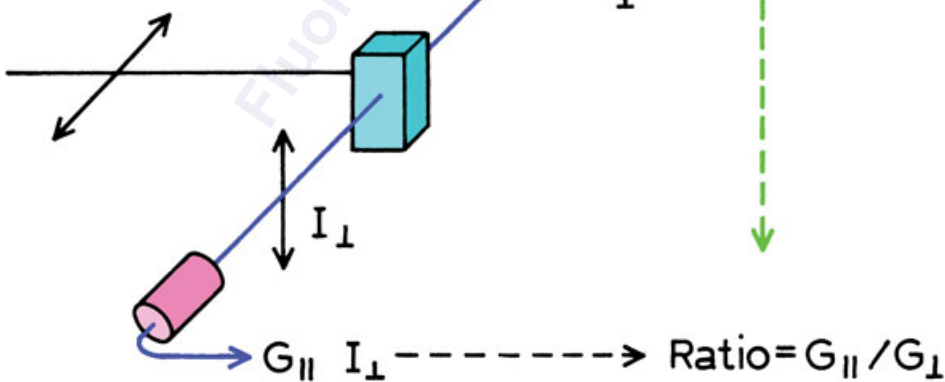




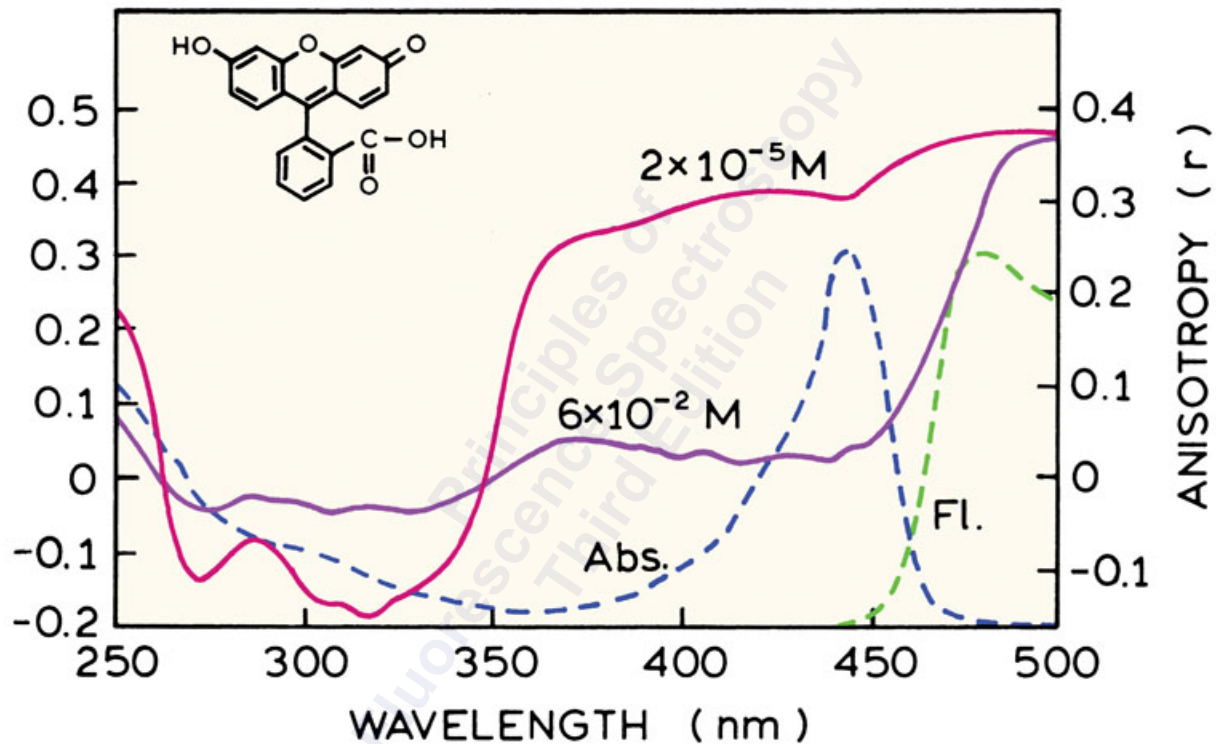
Vertical  
Excitation



Horizontal  
Excitation



POLARIZATION (P)



TEMPERATURE ( $^{\circ}\text{C}$ )

2

20

37

6

MW = 25 000

$h = 0.4$

$\tau = 4 \text{ ns}$

5

$\frac{1}{r}$

4

$r_0^{\text{app}} = 0.3$

3

Slope =  $\frac{\tau R}{r_0 V}$ ,

$r_0 = 0.4$

$\frac{1}{r_0}$

2

0 10 20 30 40 50  $\times 10^3$

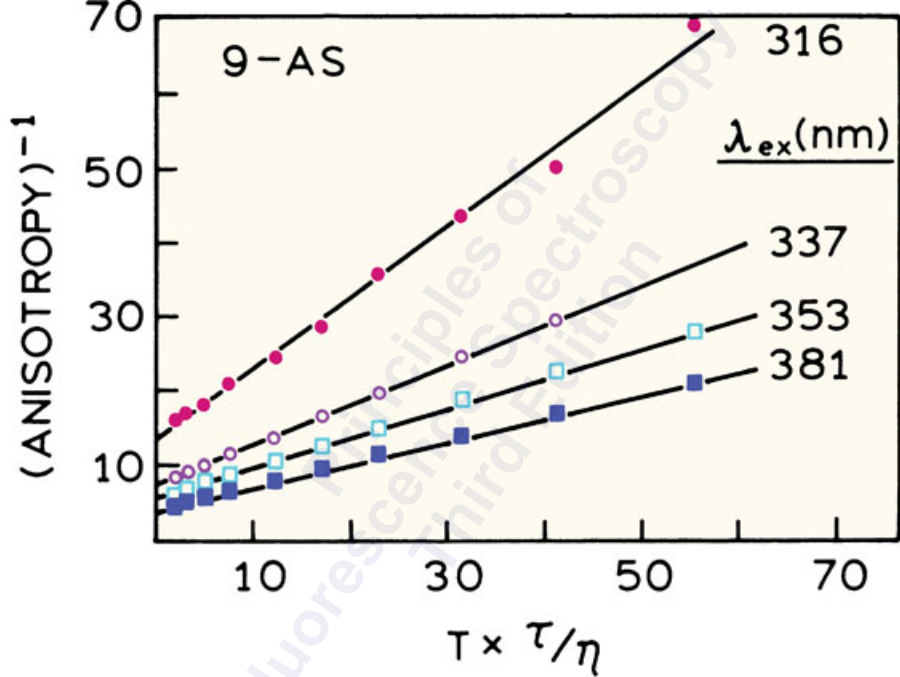
$T/\eta$  ( $^{\circ}\text{K} \times \text{P}^{-1}$ )

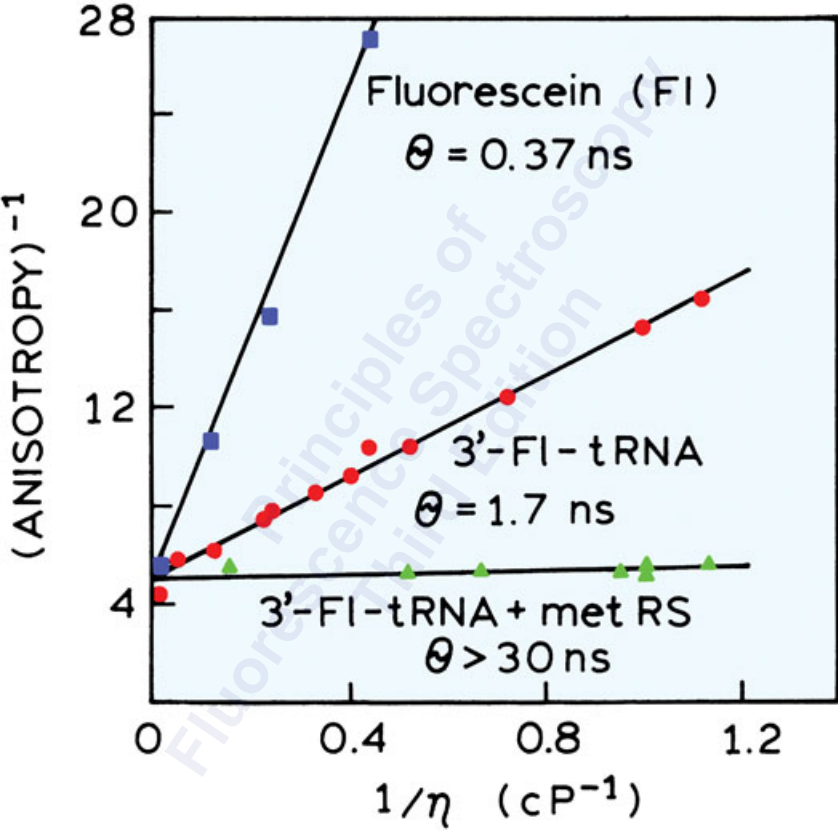
$\frac{1}{r}$

Slope =  $\frac{\tau R}{r_0 V}$ ,

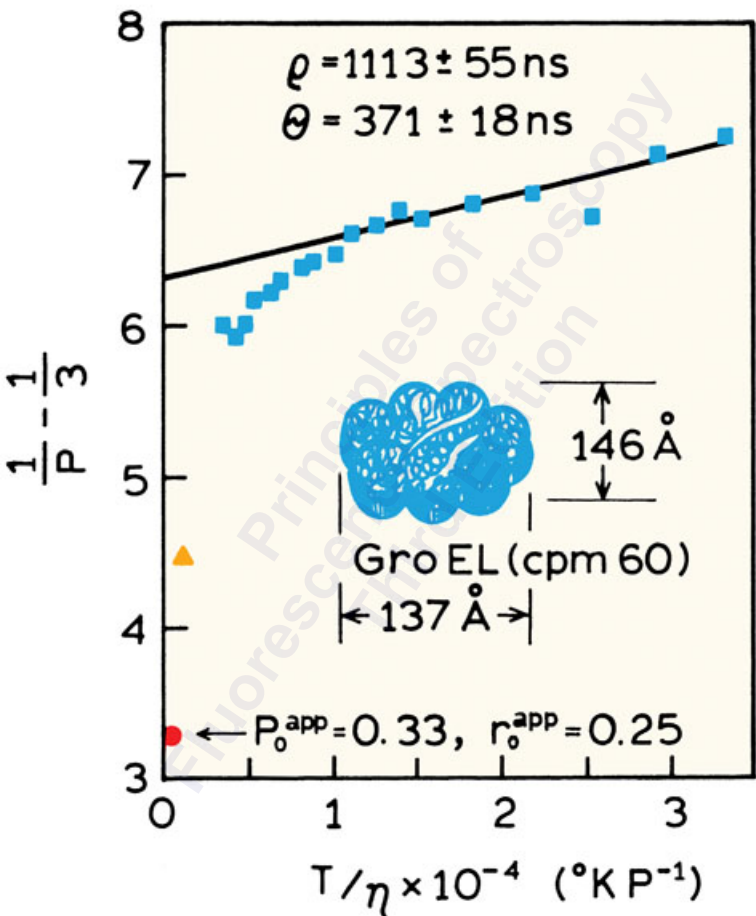
$r_0 = 0.4$

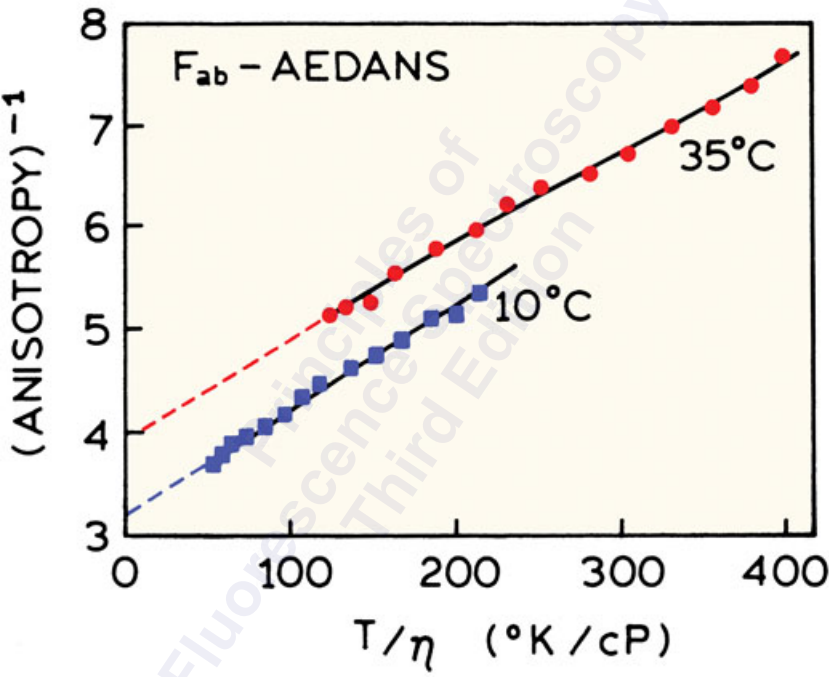
$\frac{1}{r_0}$









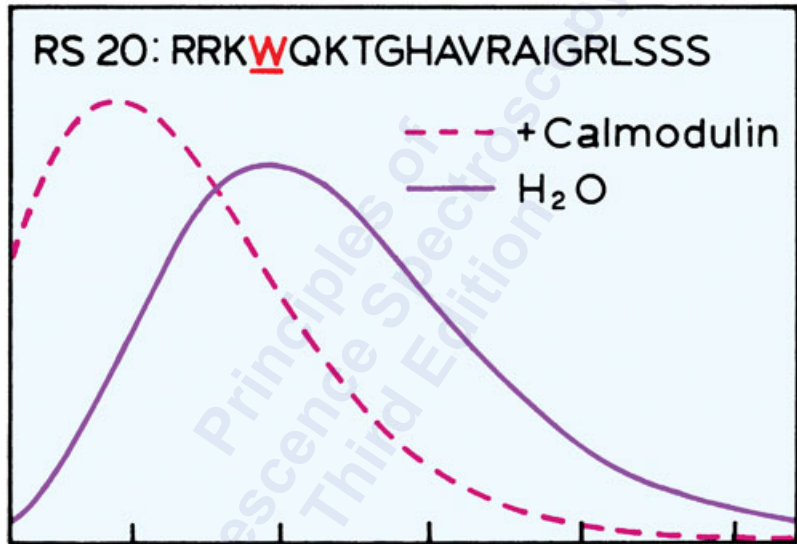


FLUORESCENCE INTENSITY

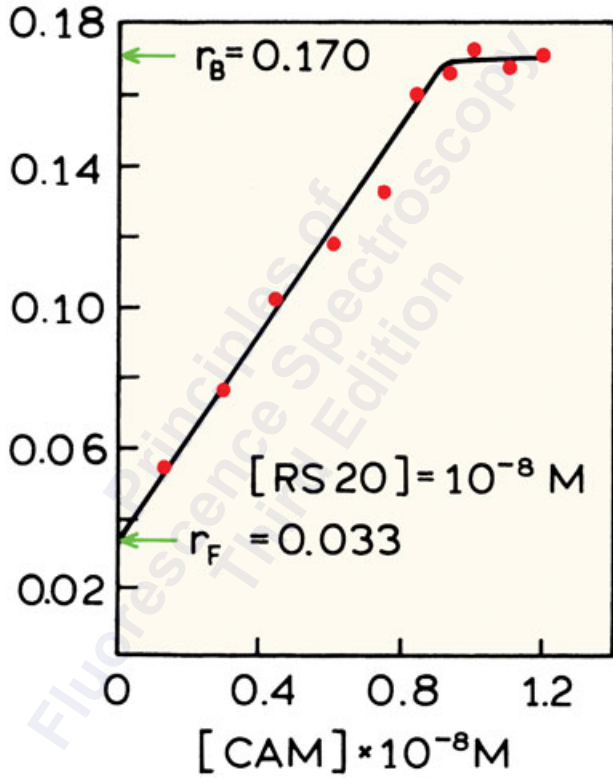
RS 20: RRKWQKTGHAVRAIGRLSSS

--- + Calmodulin  
— H<sub>2</sub>O

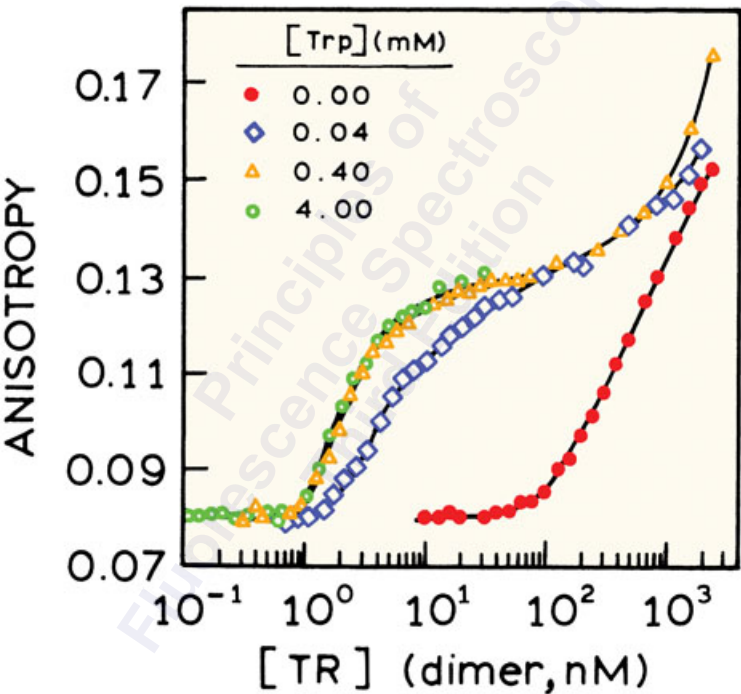
325 350 375 400 425  
WAVELENGTH ( nm )

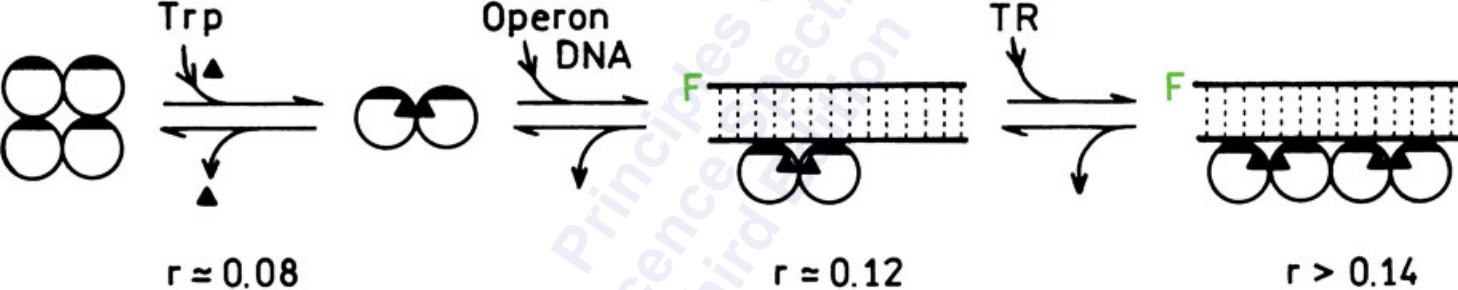


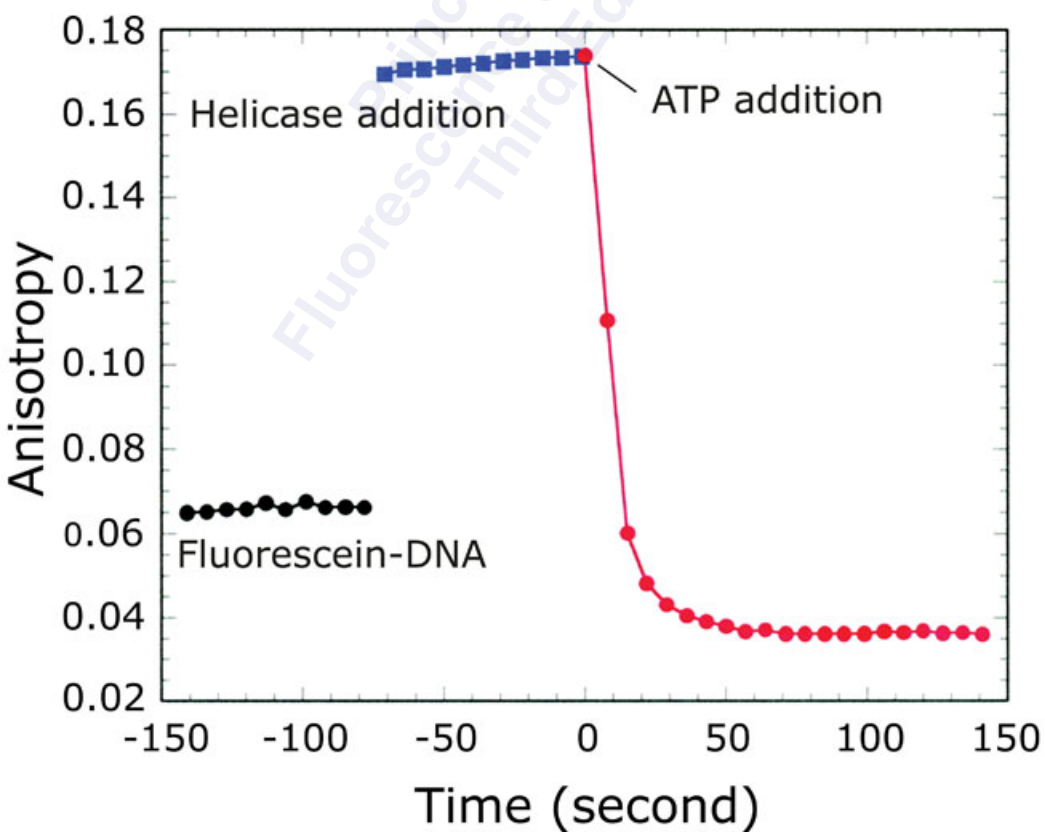
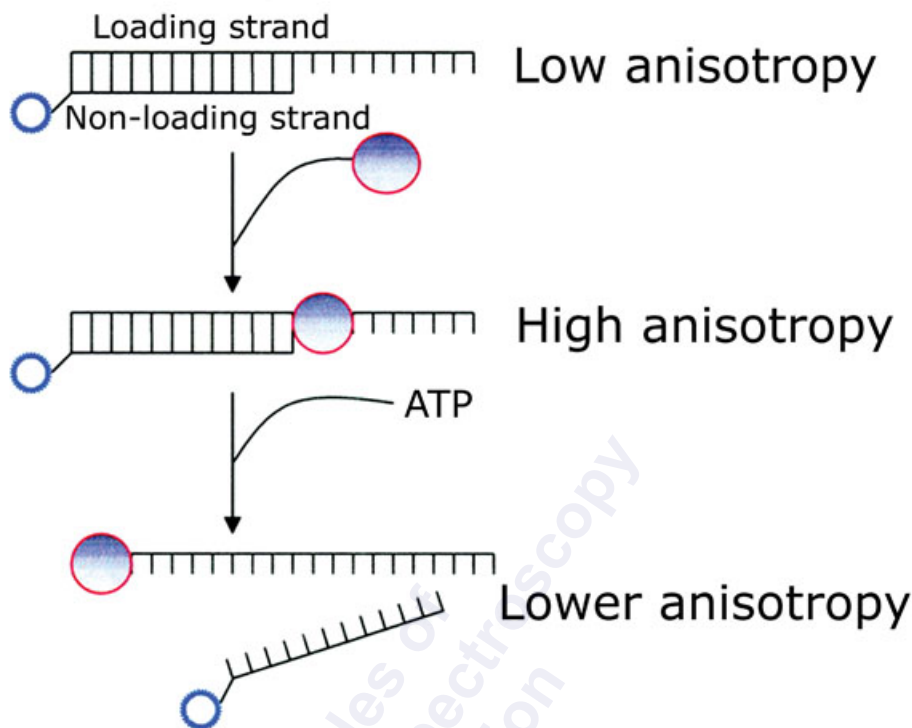
ANISOTROPY

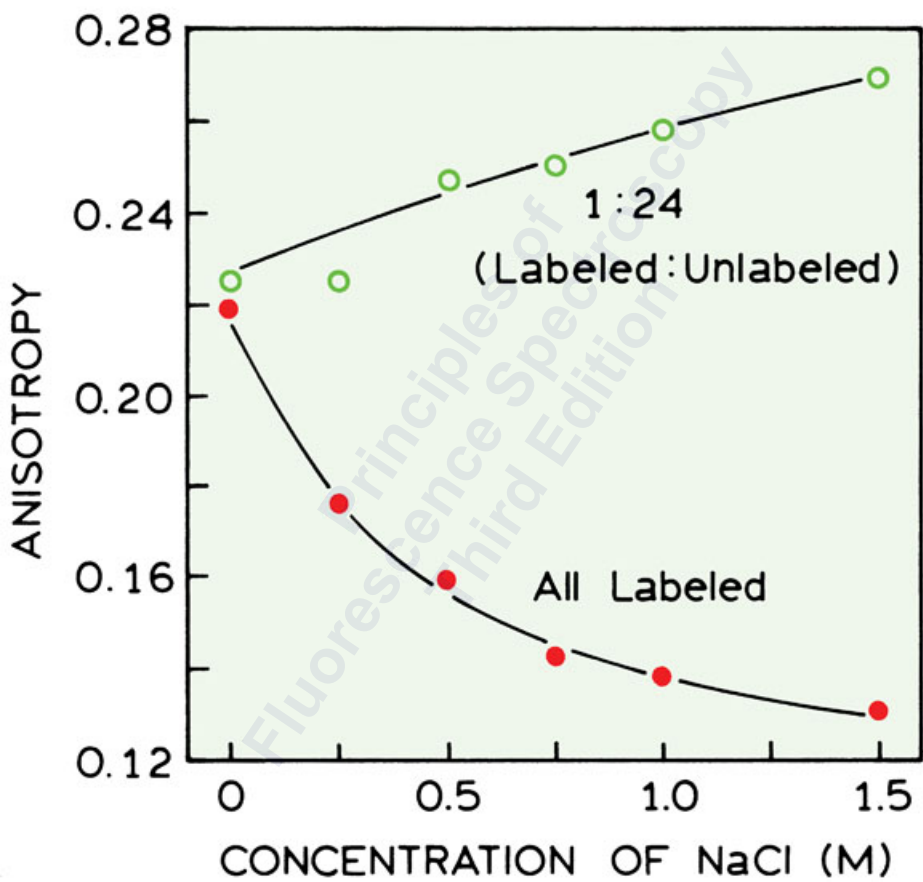


5 **F**ATCGAACTAGTTAACTAGTACGCAA 3  
 |||||  
 3 TAGCTTGATCAATTGATCATGCGTT 5



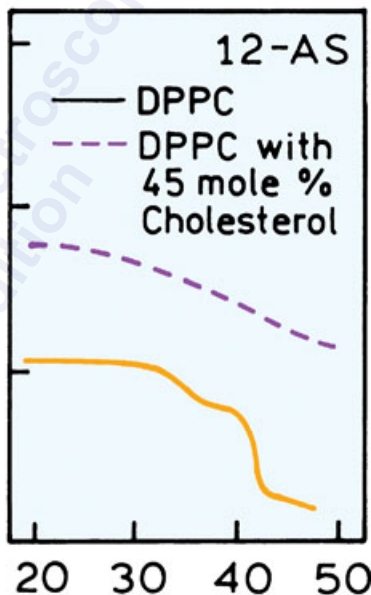
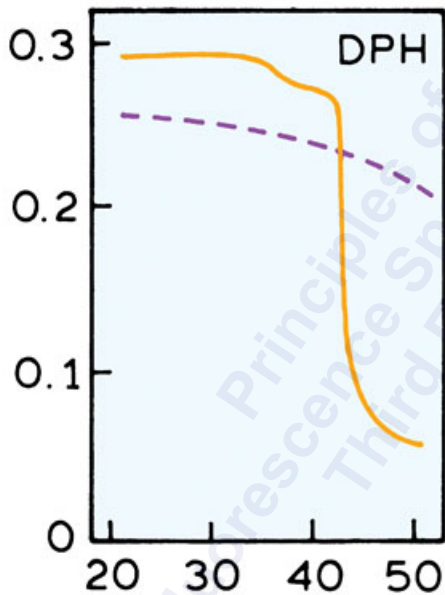






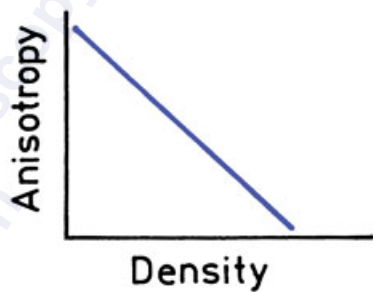
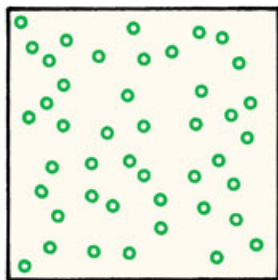


ANISOTROPY

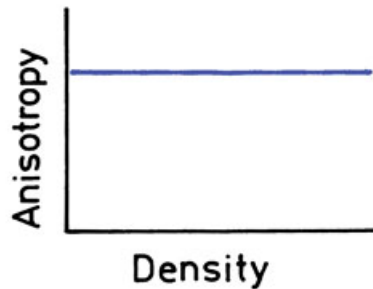
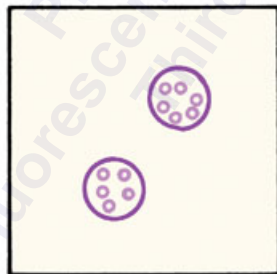
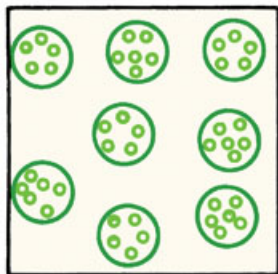


TEMPERATURE (°C)

## Random distribution



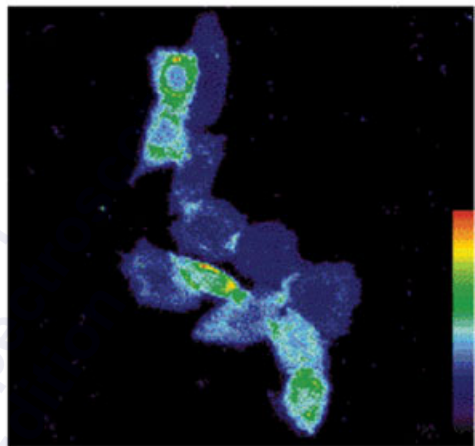
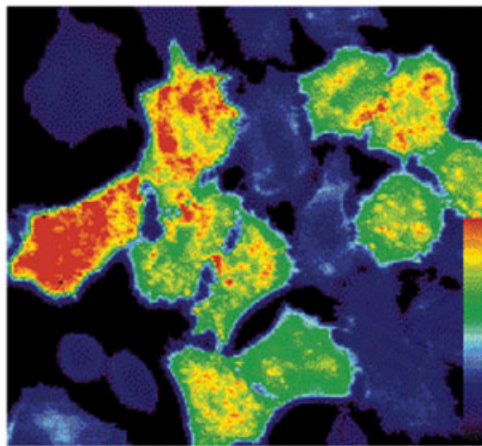
## Clustered distribution



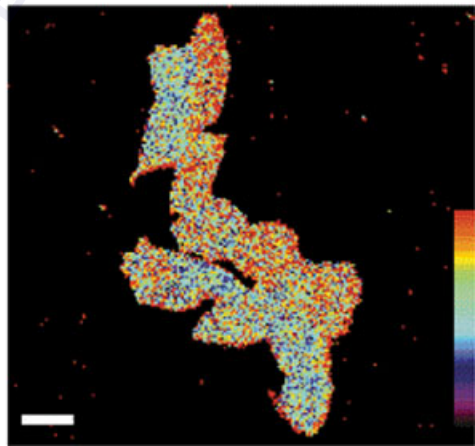
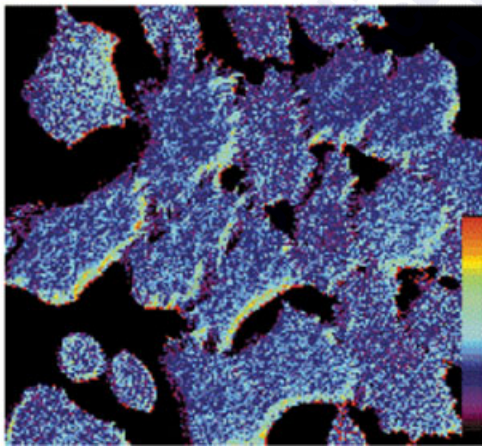
FR-GPI

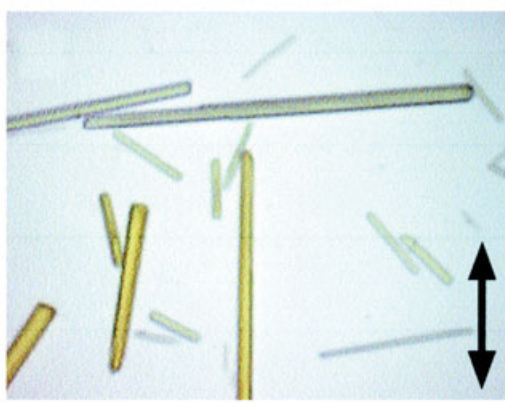
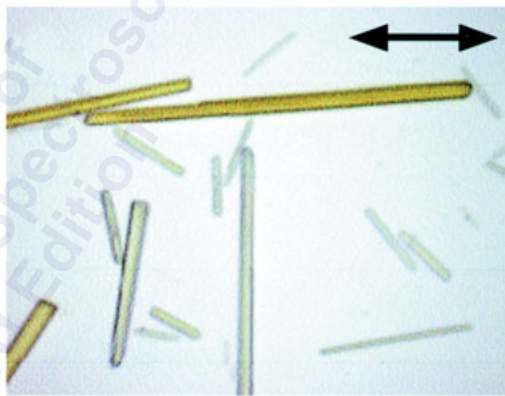
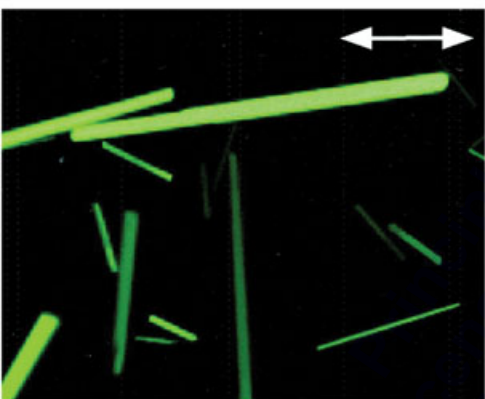
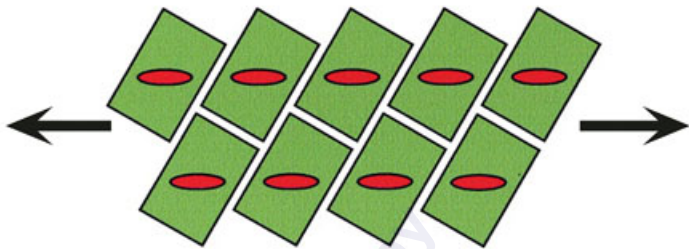
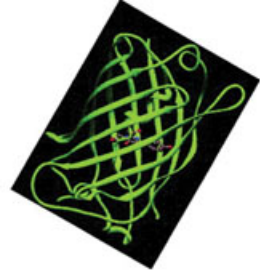
FR-TM

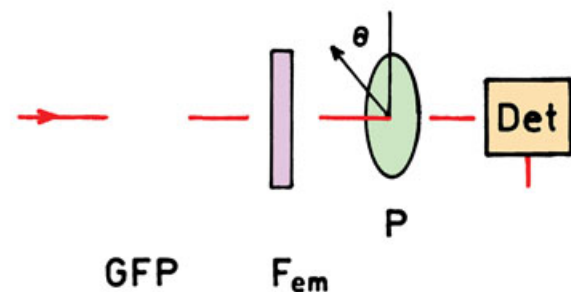
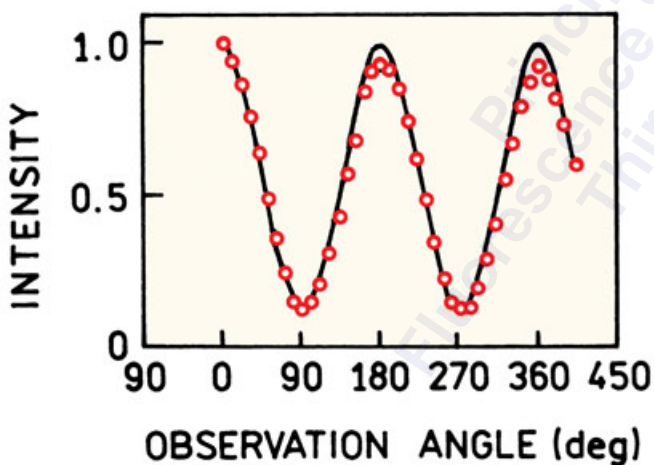
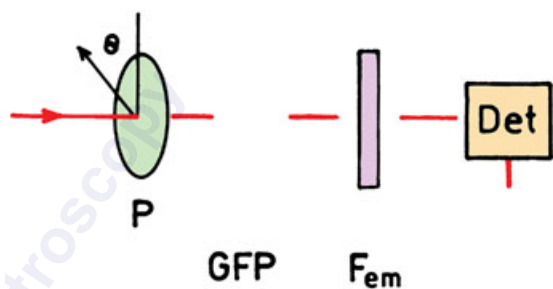
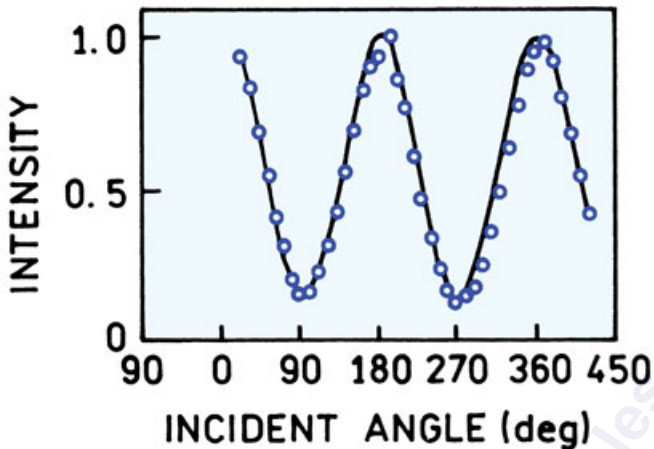
Intensity

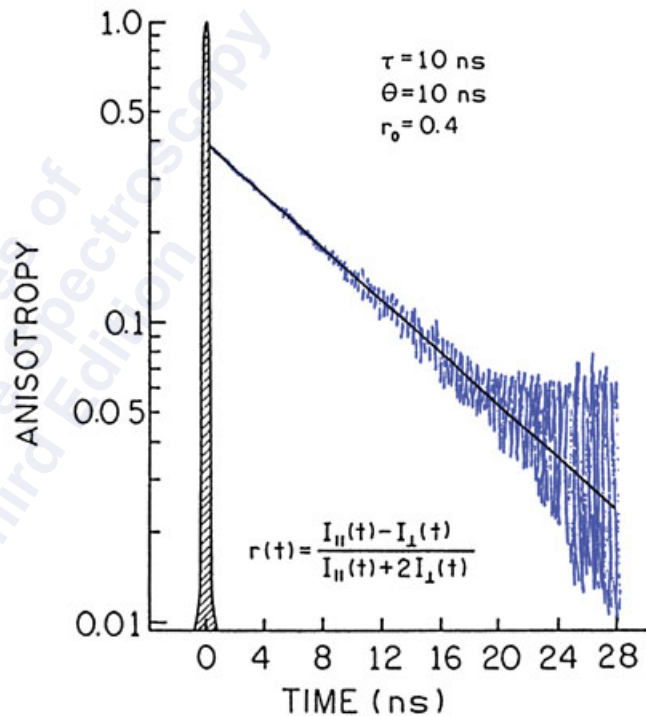
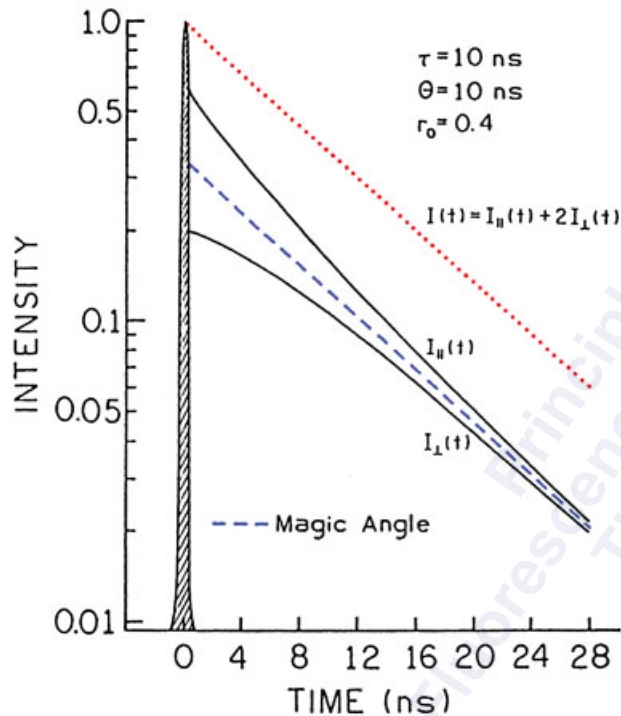


Anisotropy

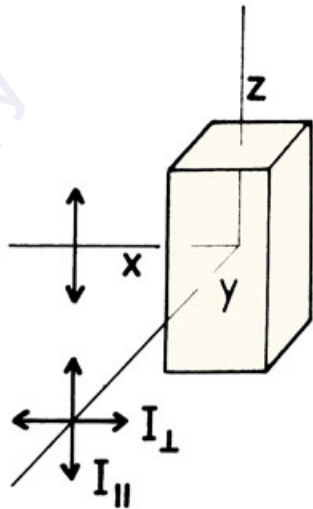
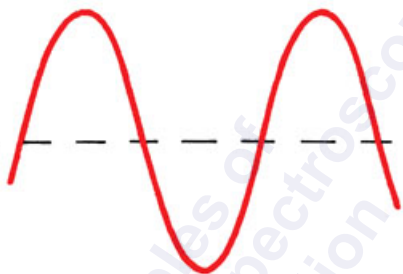




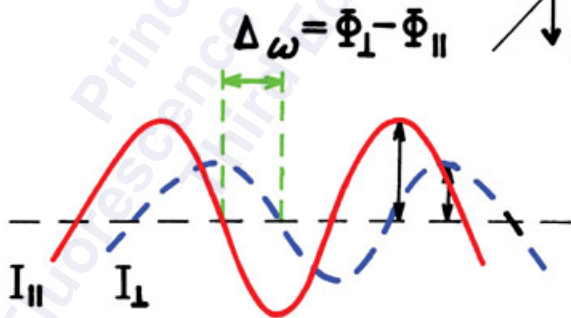




EXCITATION

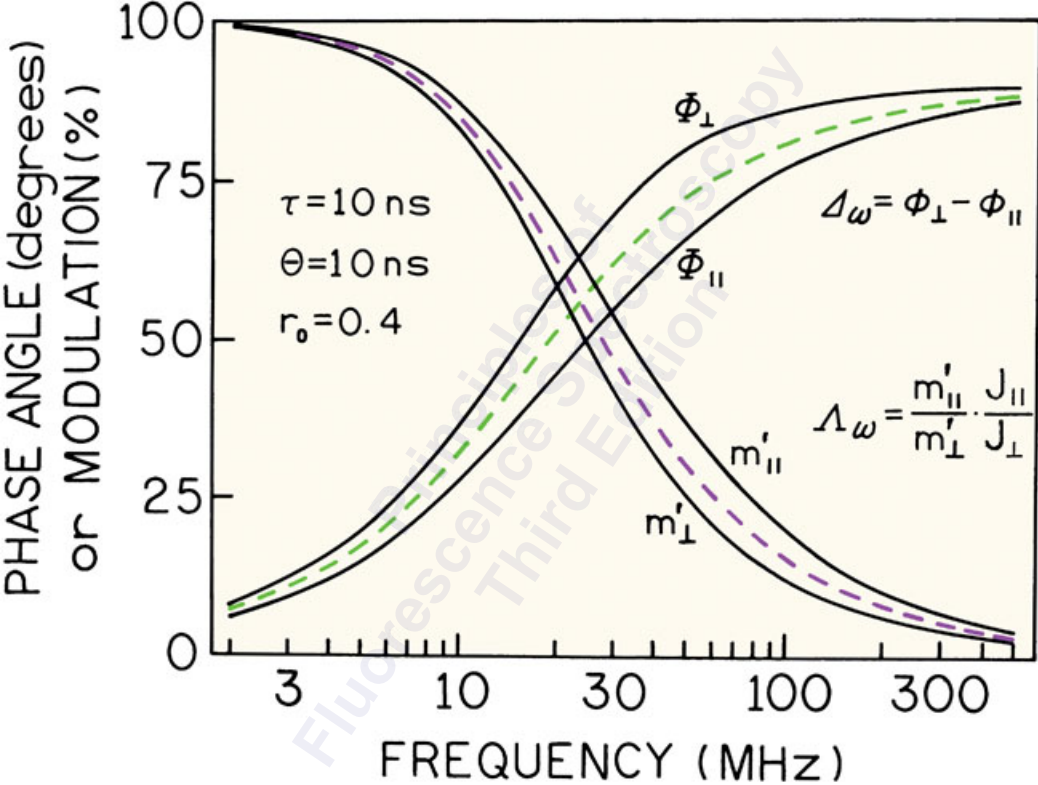


EMISSION

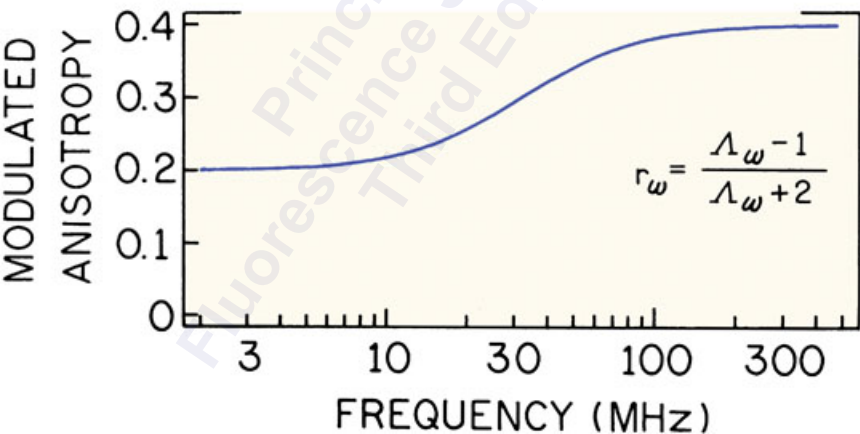
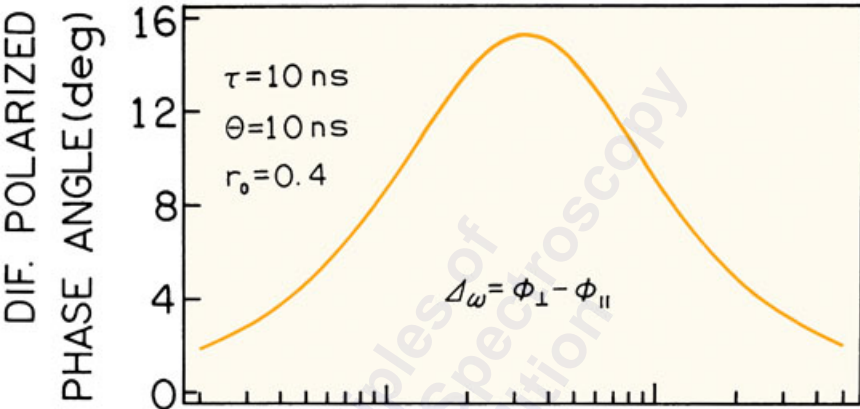


$$\Lambda_{\omega} = \frac{m_{\parallel}}{m_{\perp}}$$

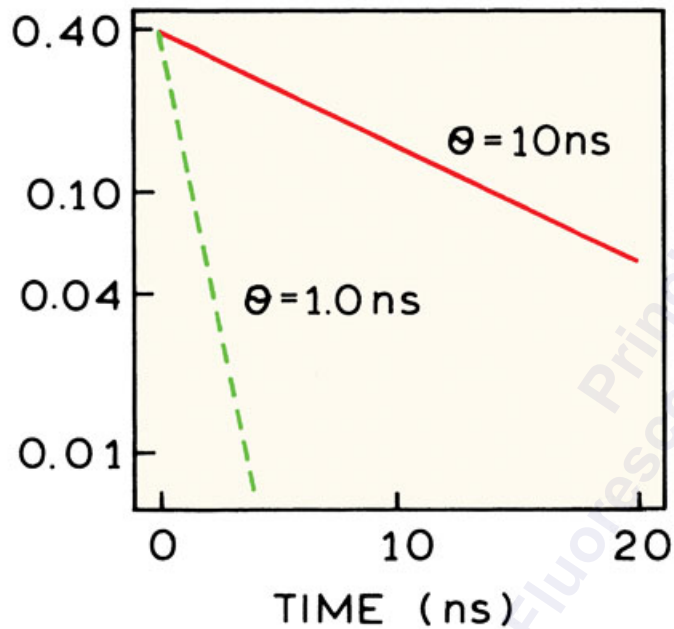
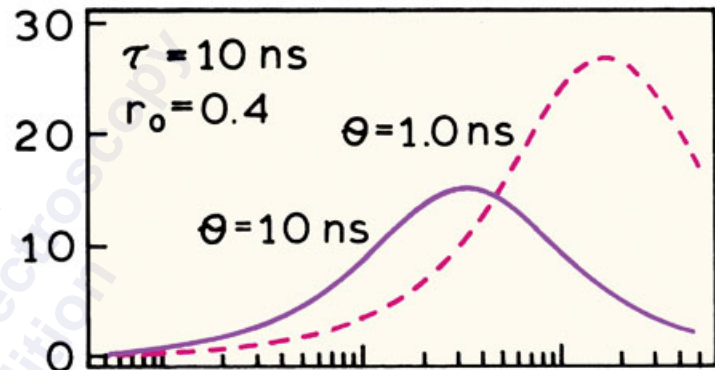
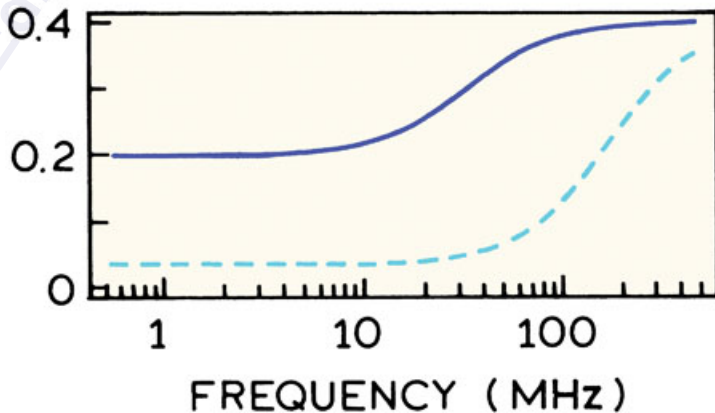




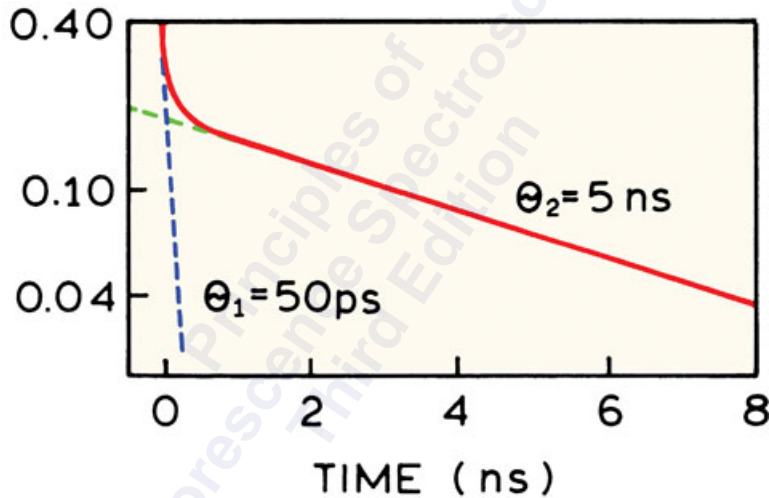


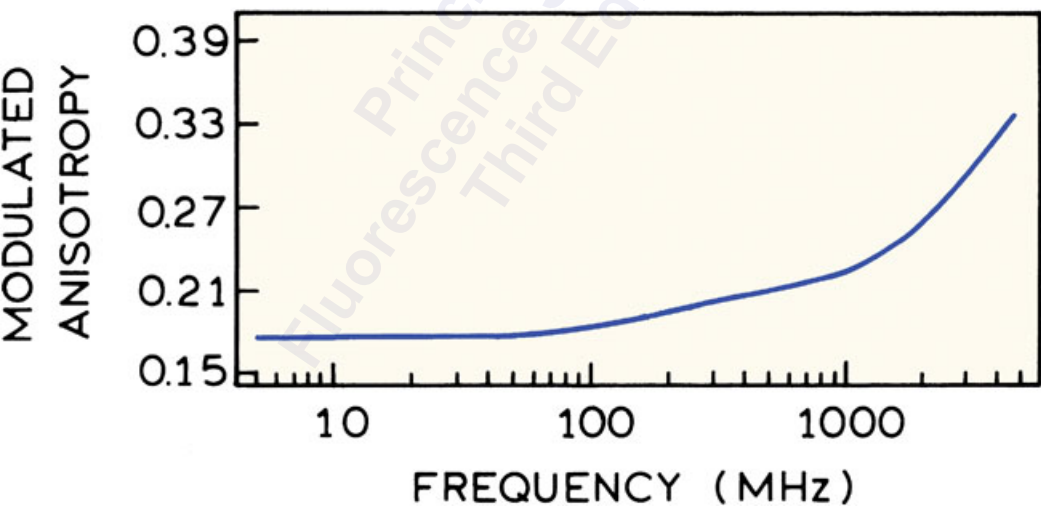
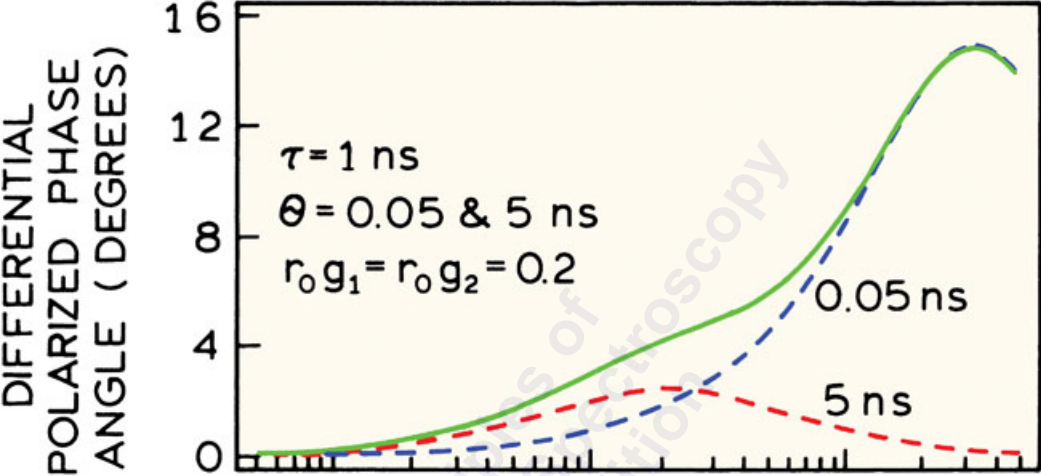


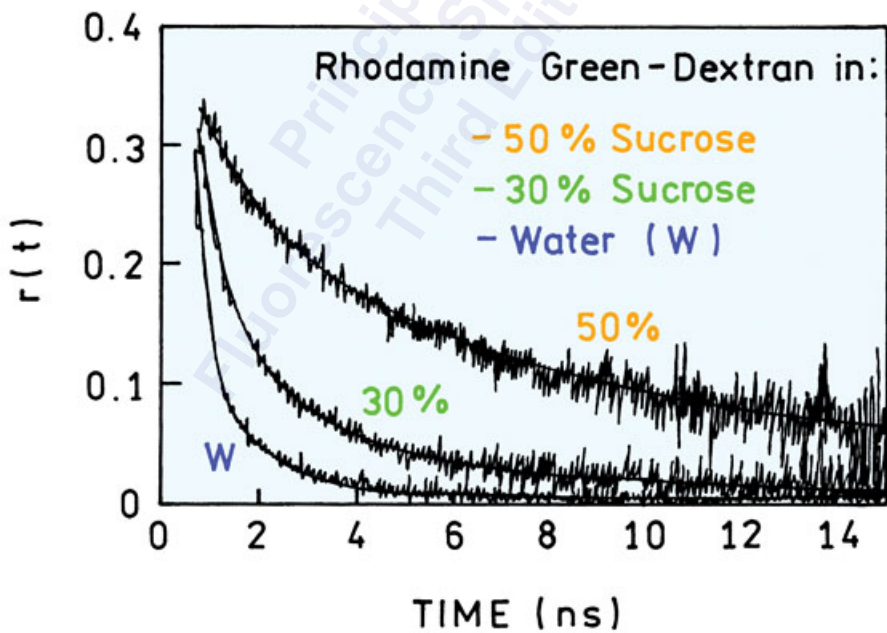
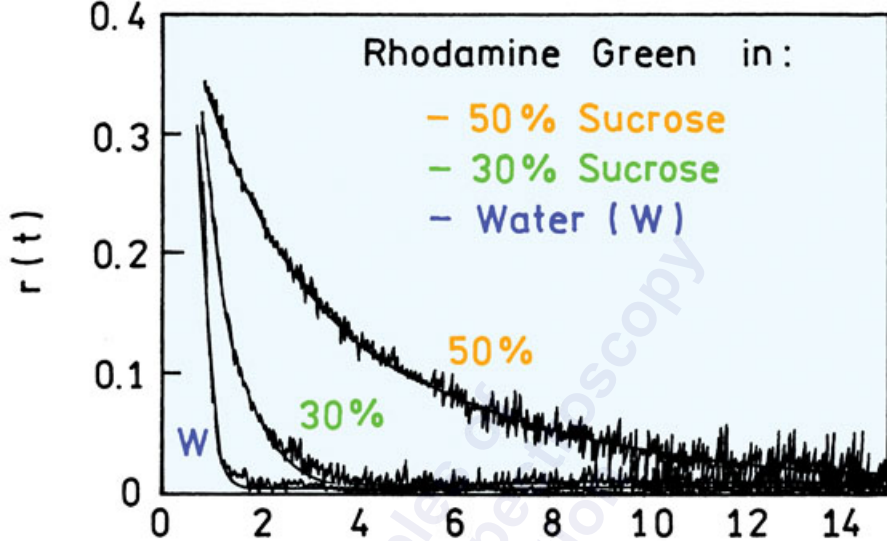
ANISOTROPY

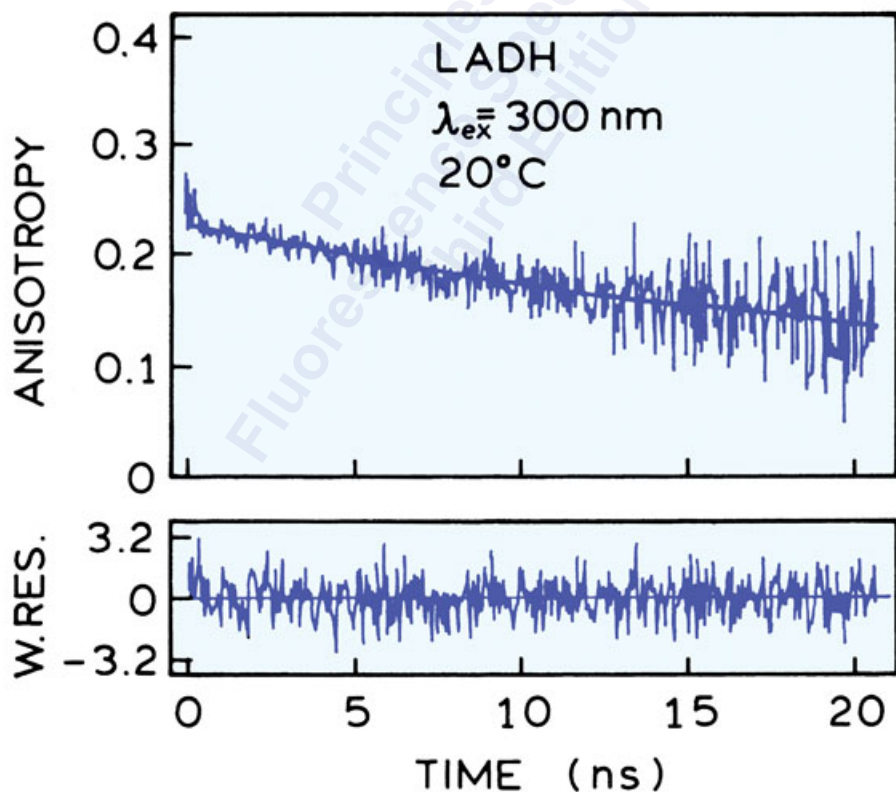
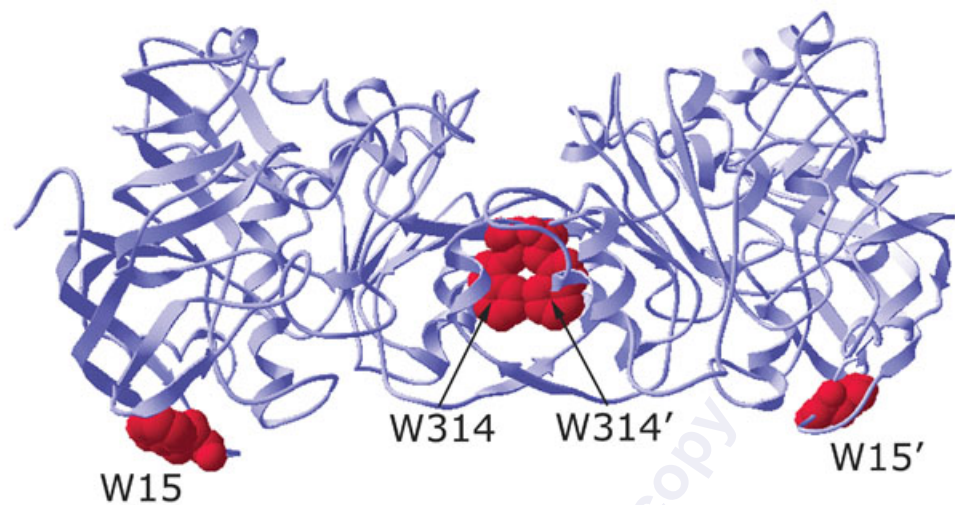
DIFFERENTIAL POLARIZED  
PHASE ANGLE (DEGREES)MODULATED  
ANISOTROPY

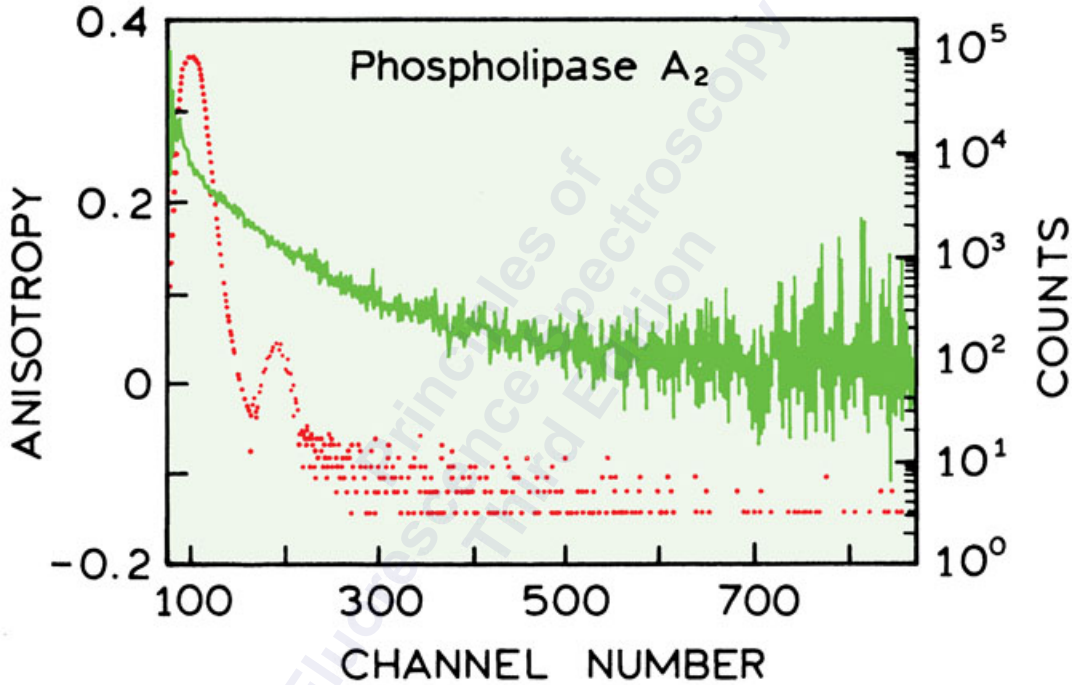
ANISOTROPY



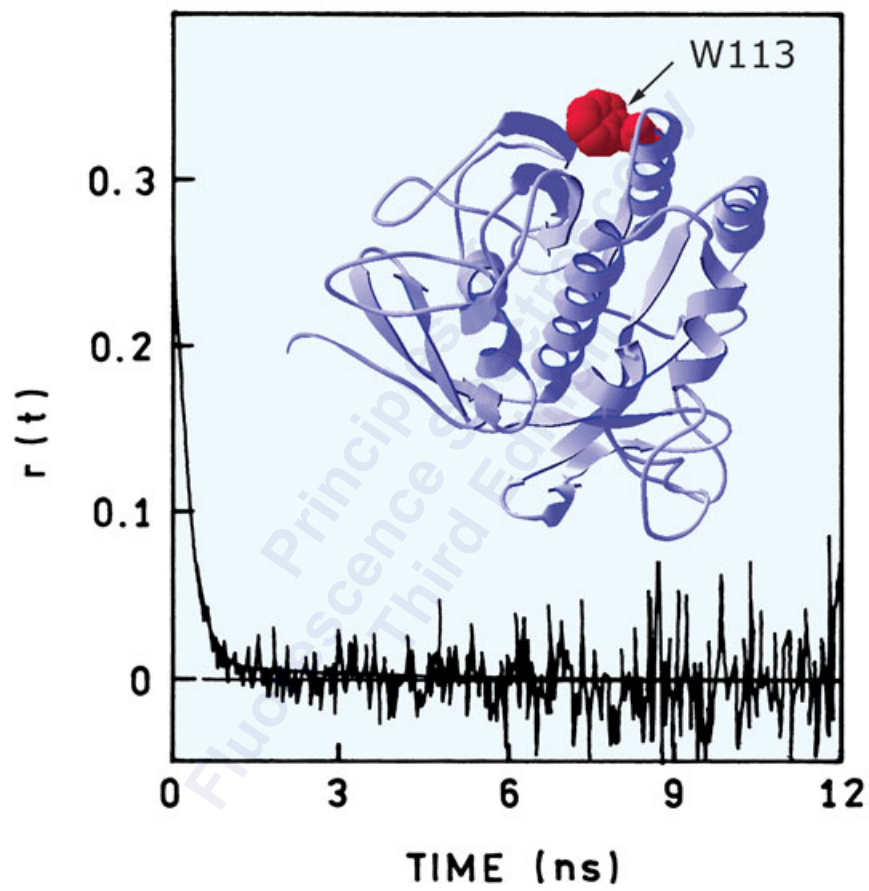




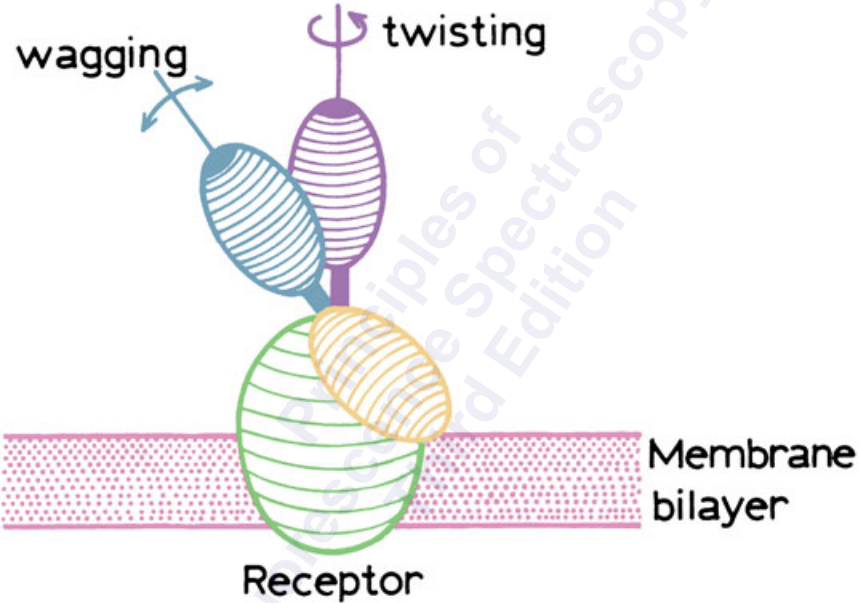


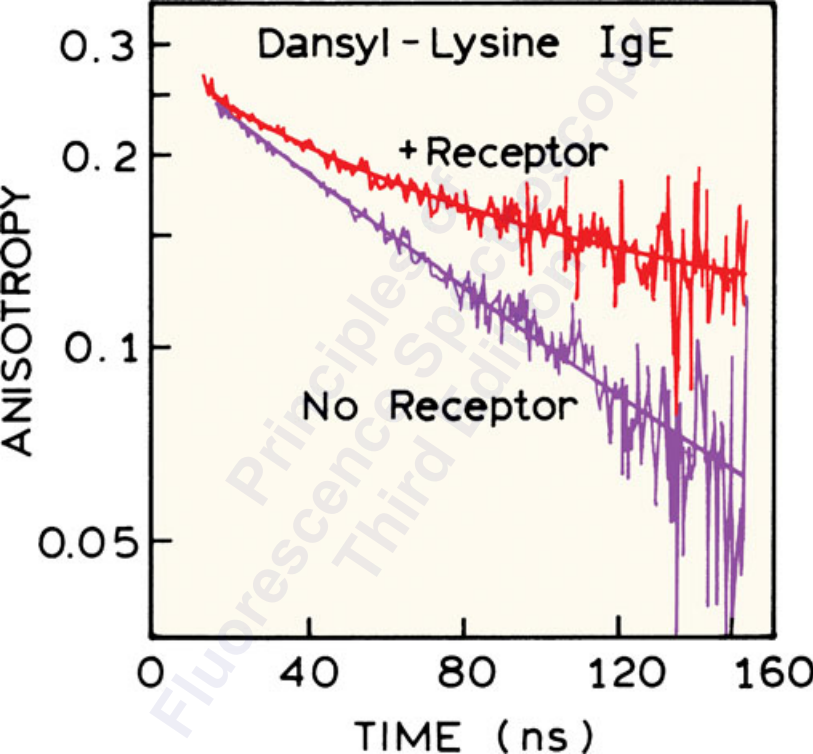


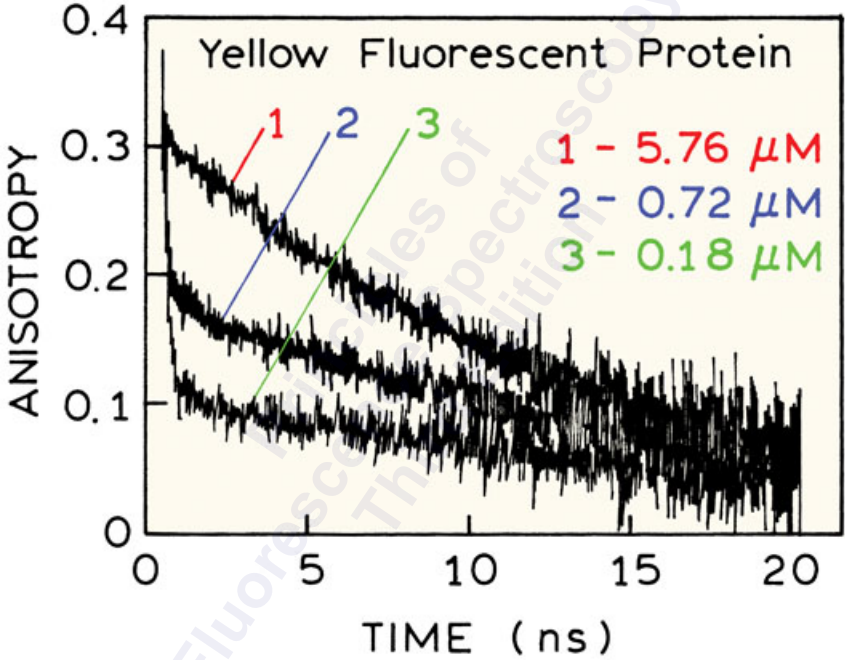




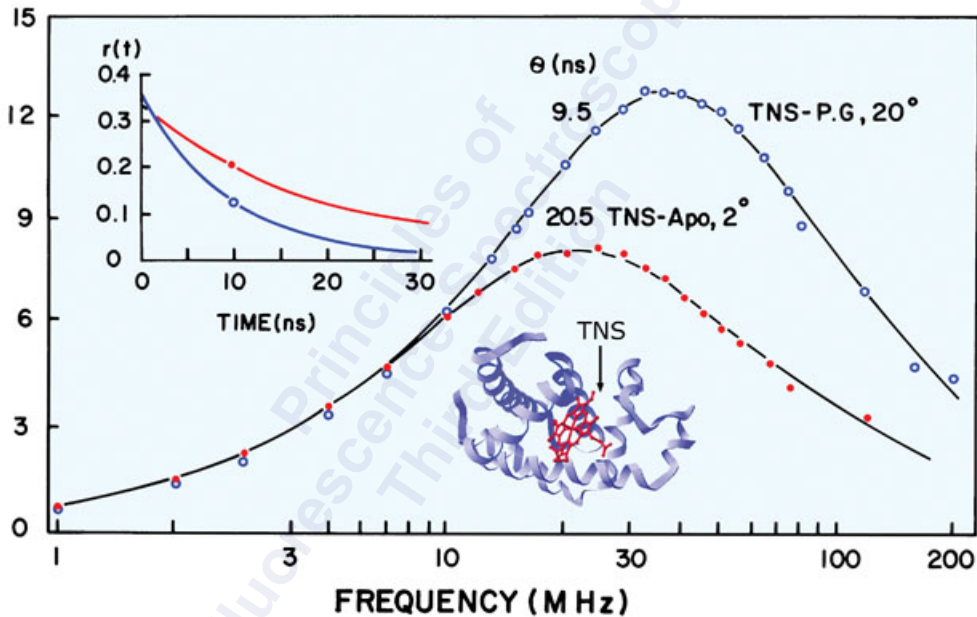


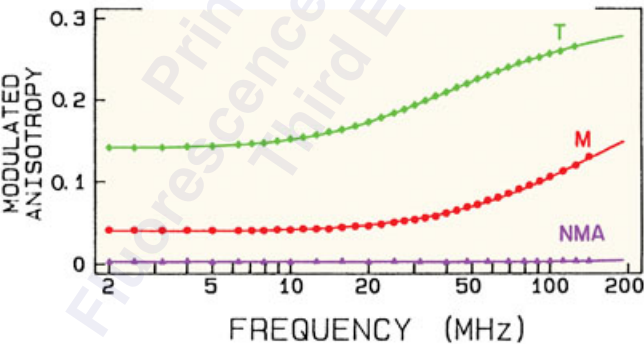
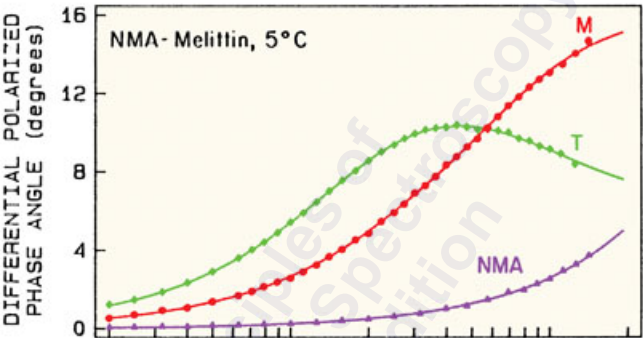


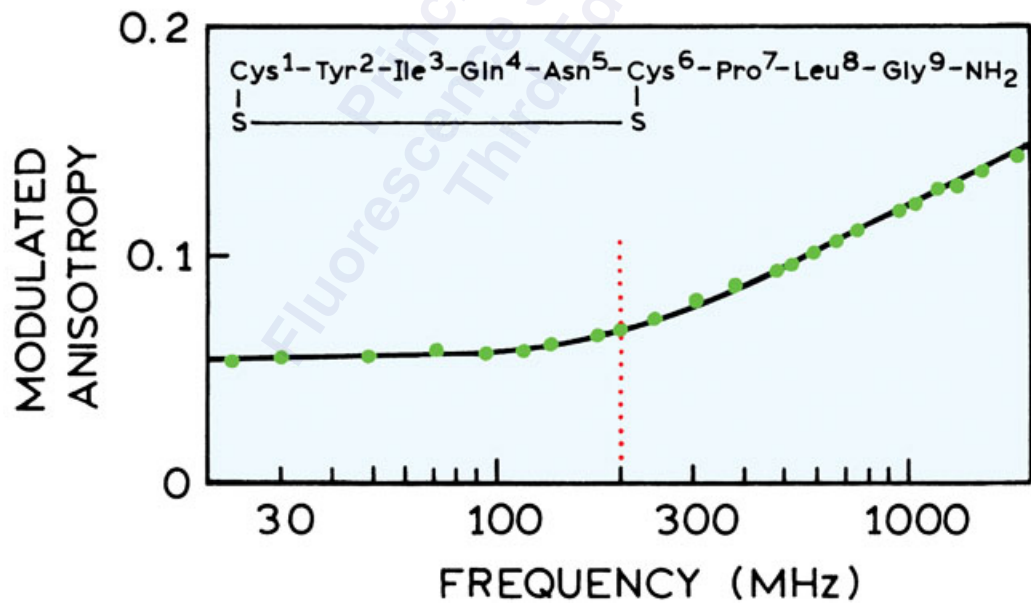
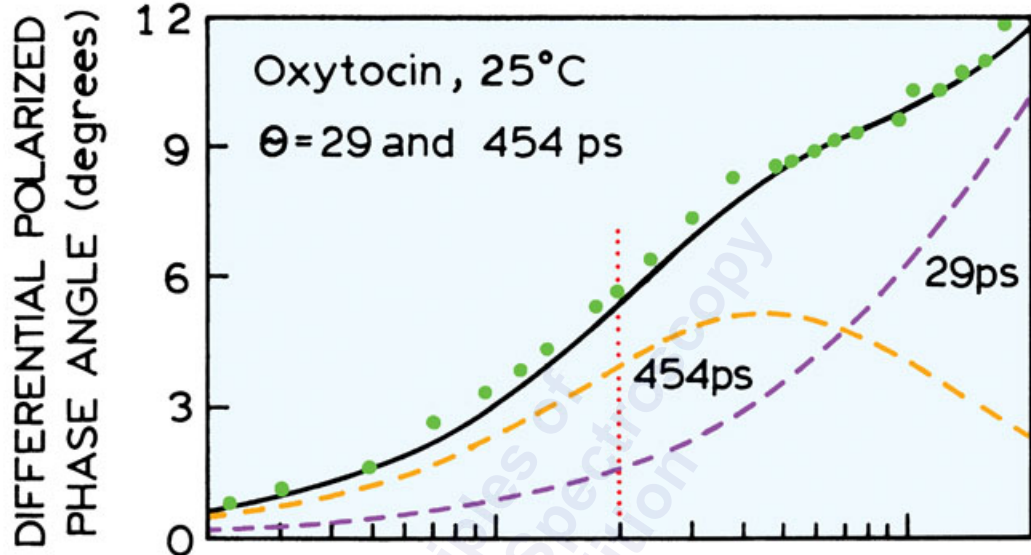


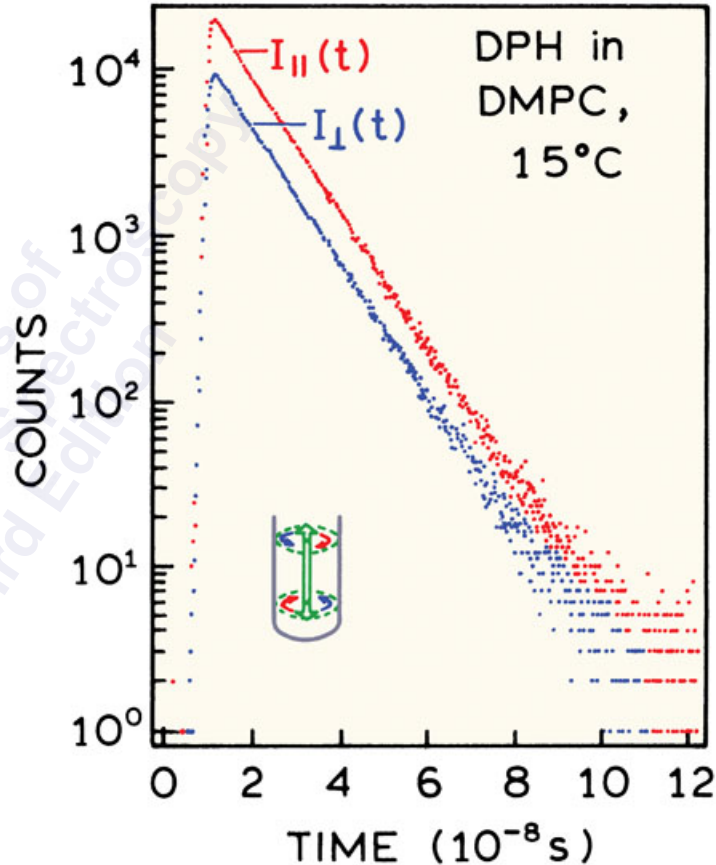
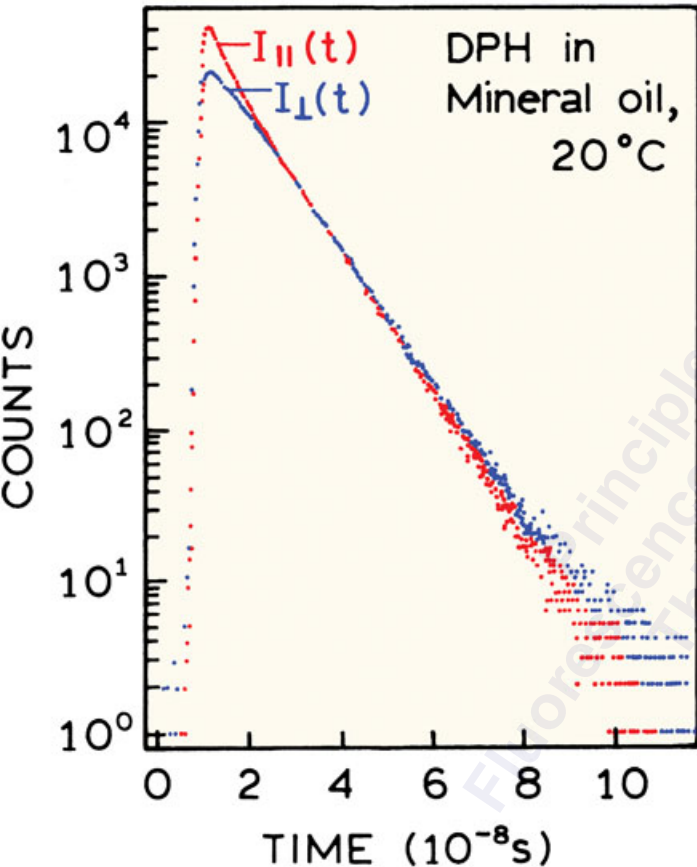


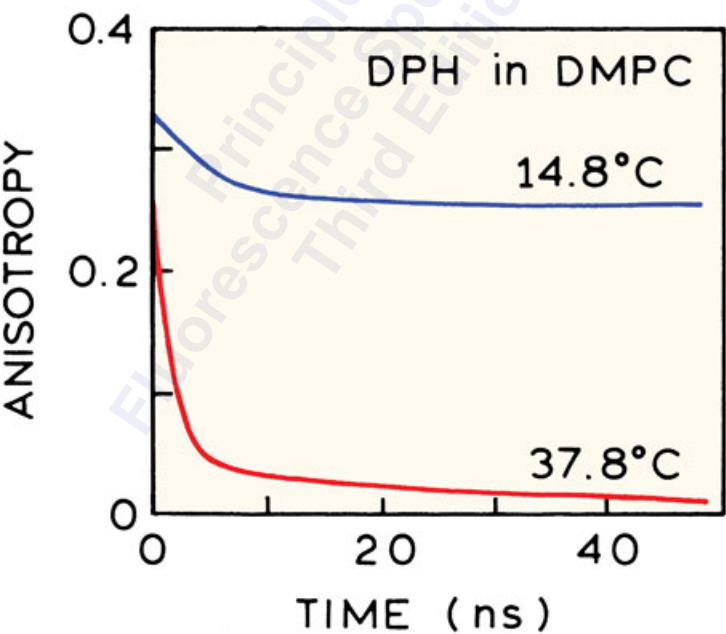
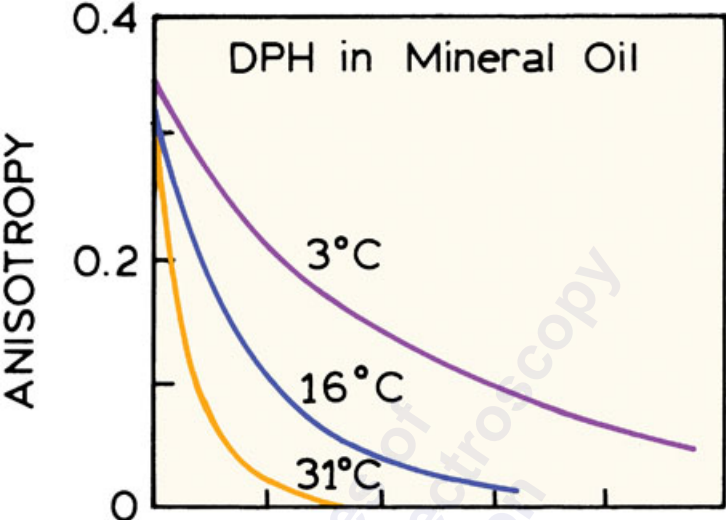
# DIFFERENTIAL POLARIZED PHASE ANGLE(degrees)





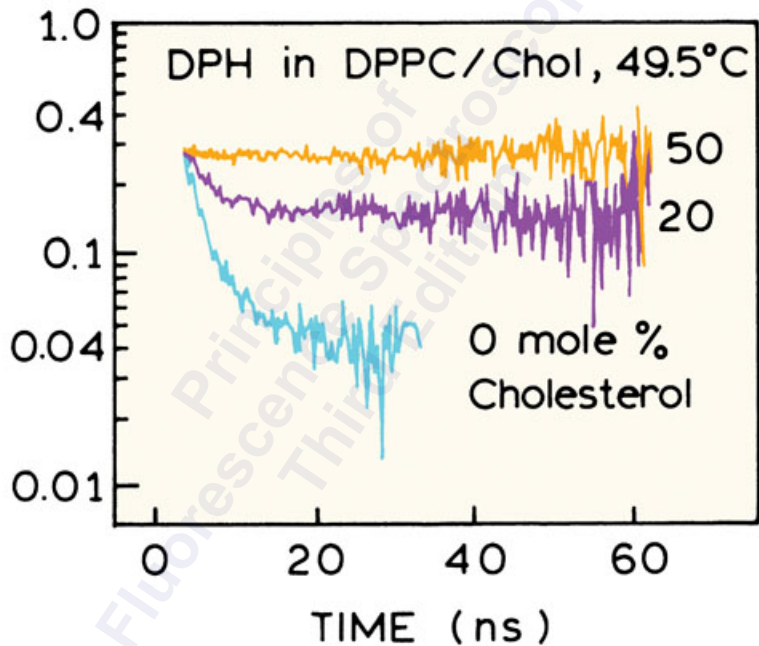


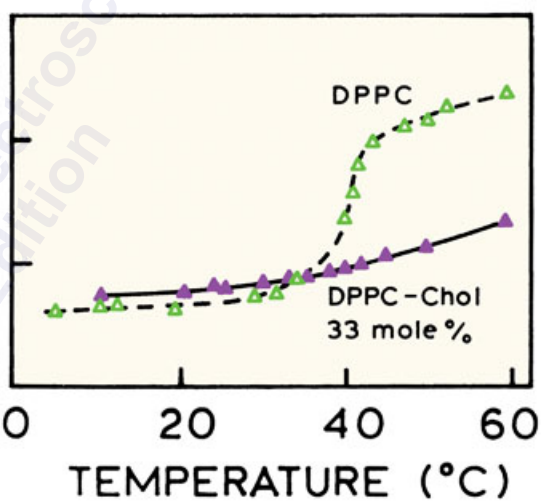
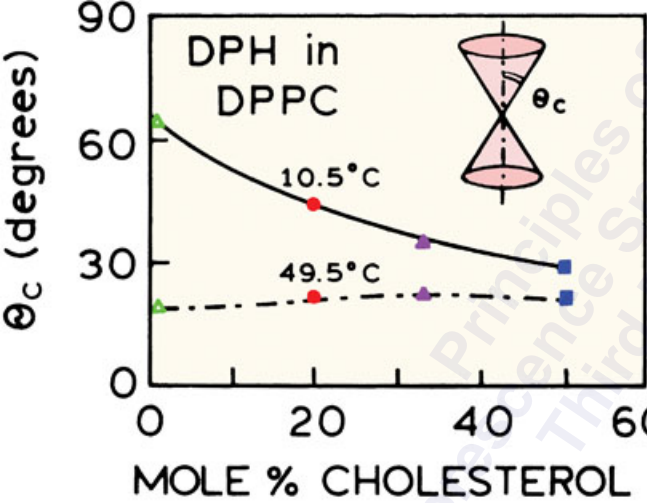


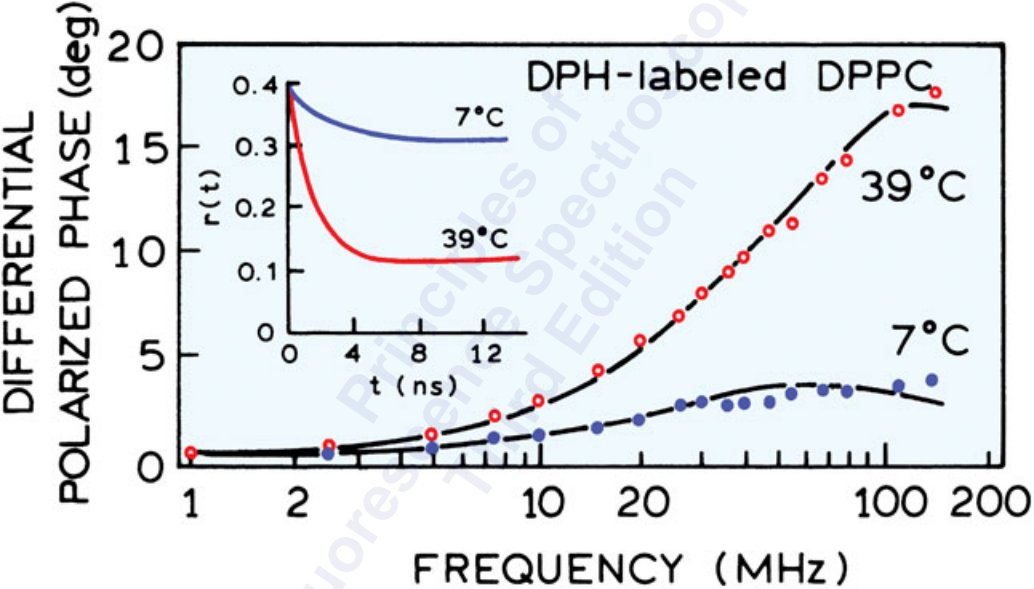


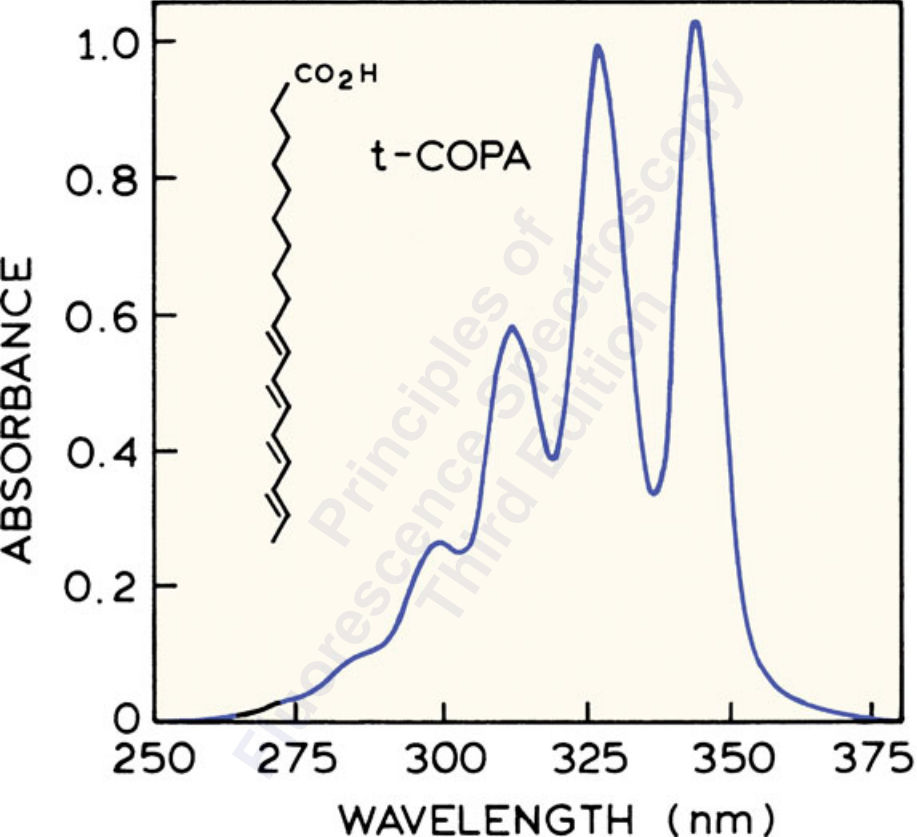


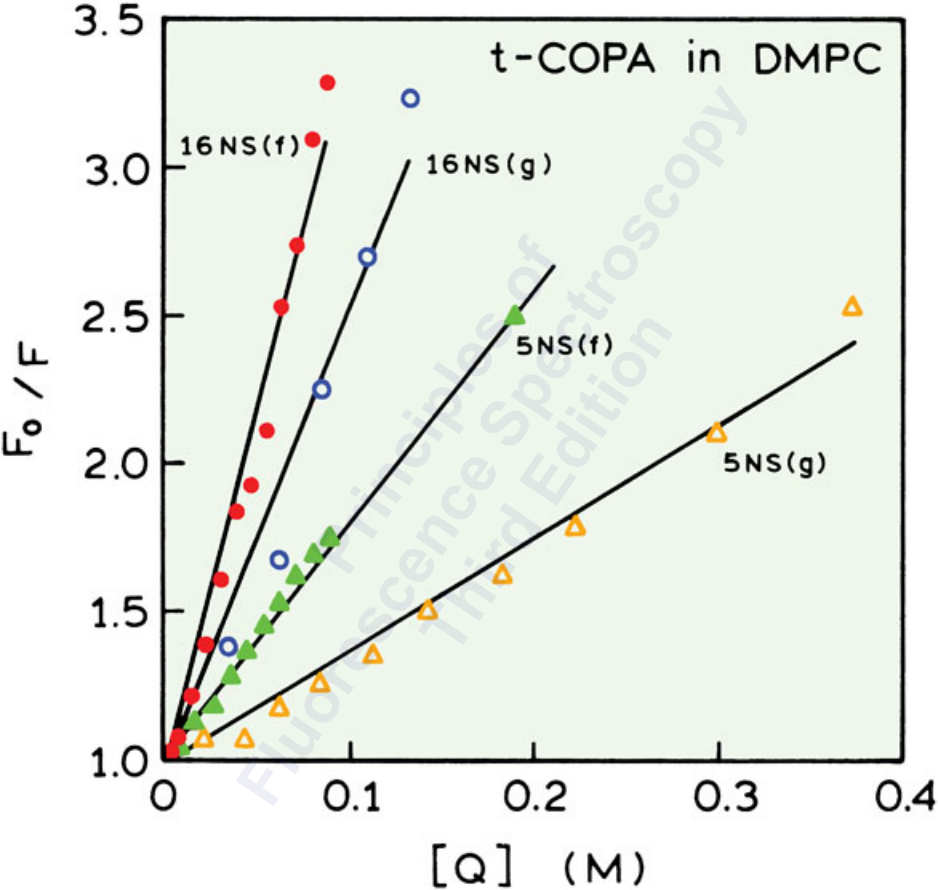
ANISOTROPY



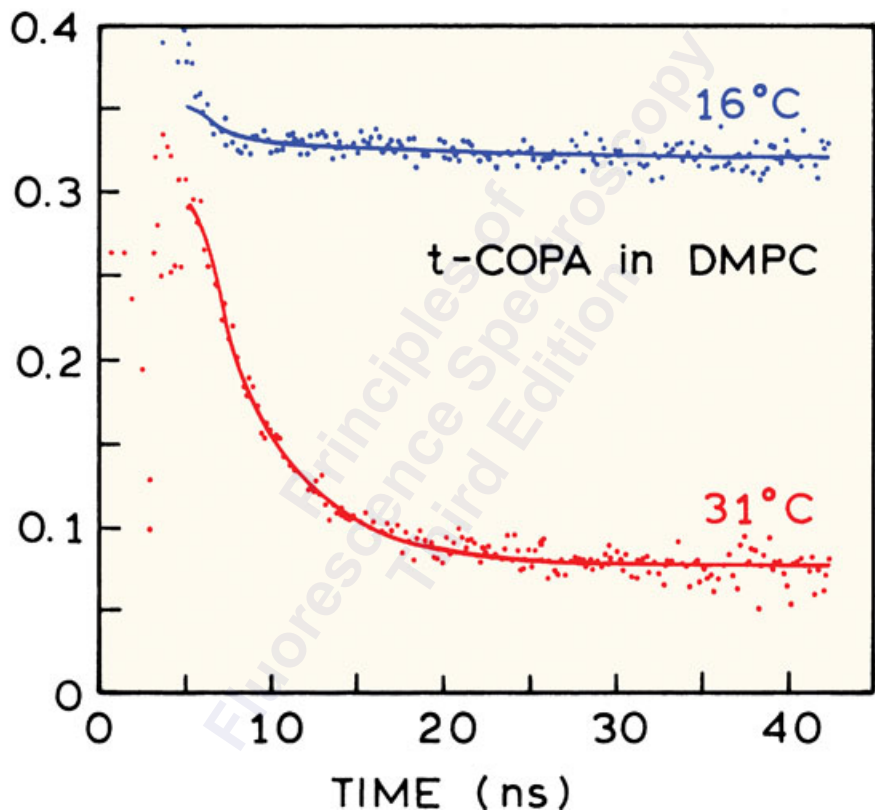


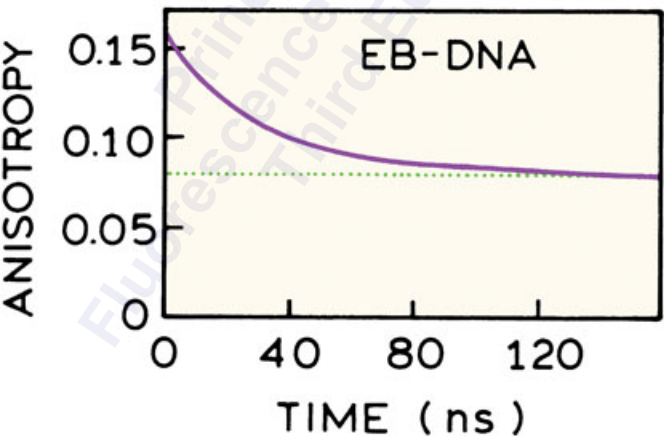
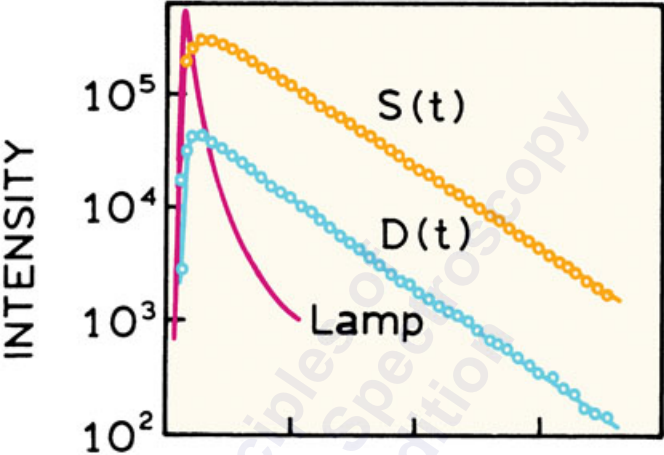


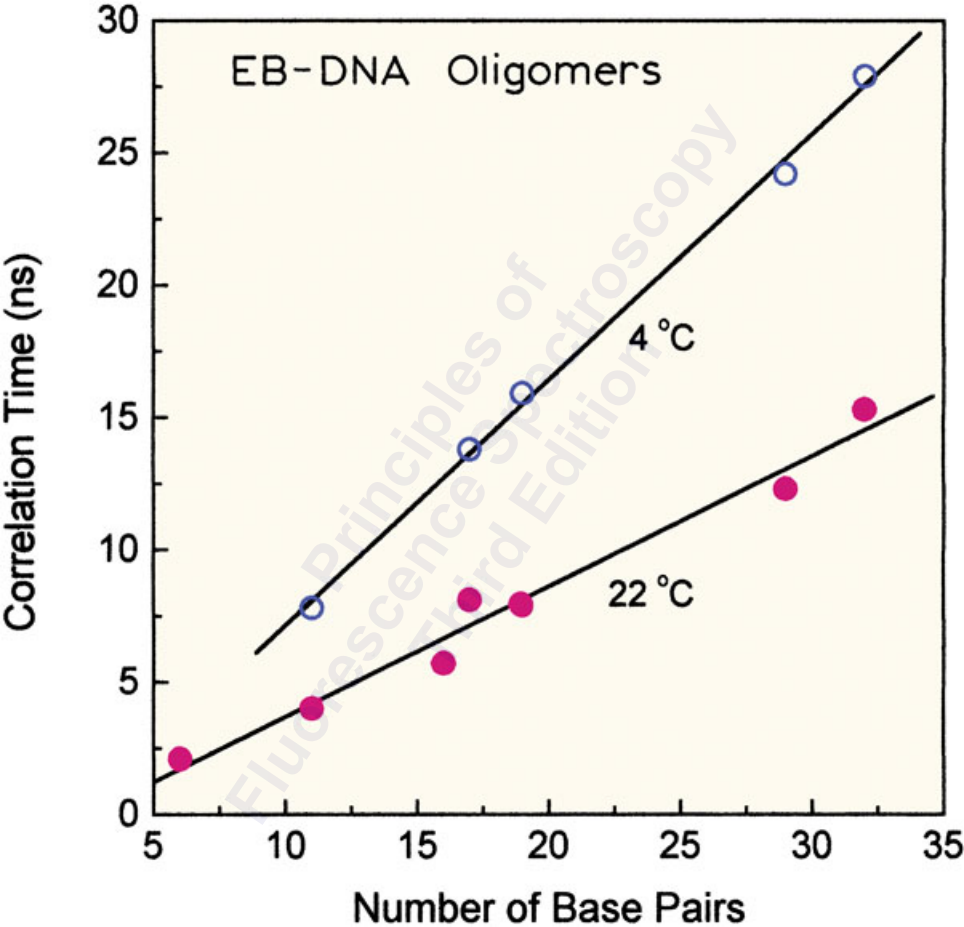




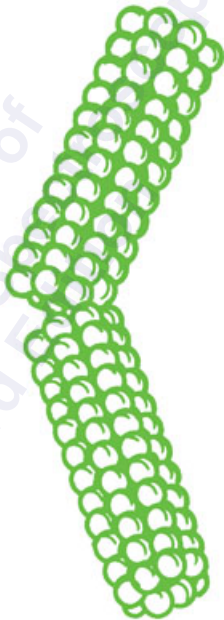
ANISOTROPY



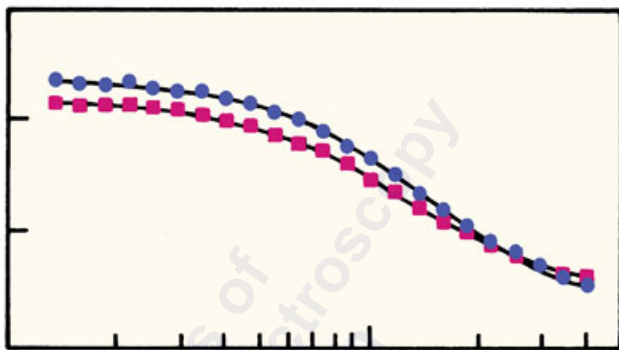




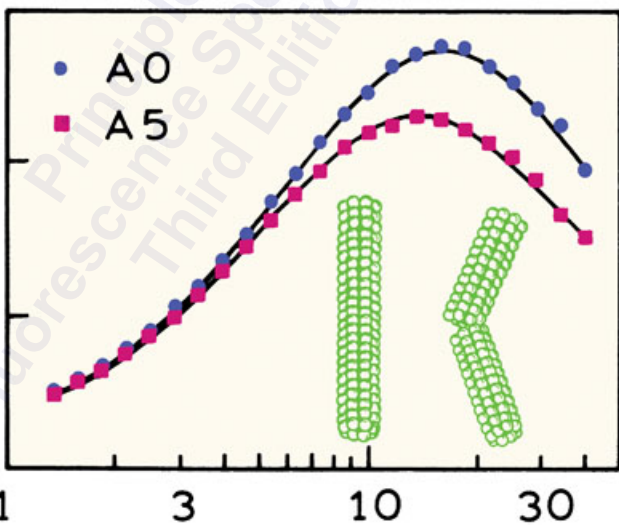




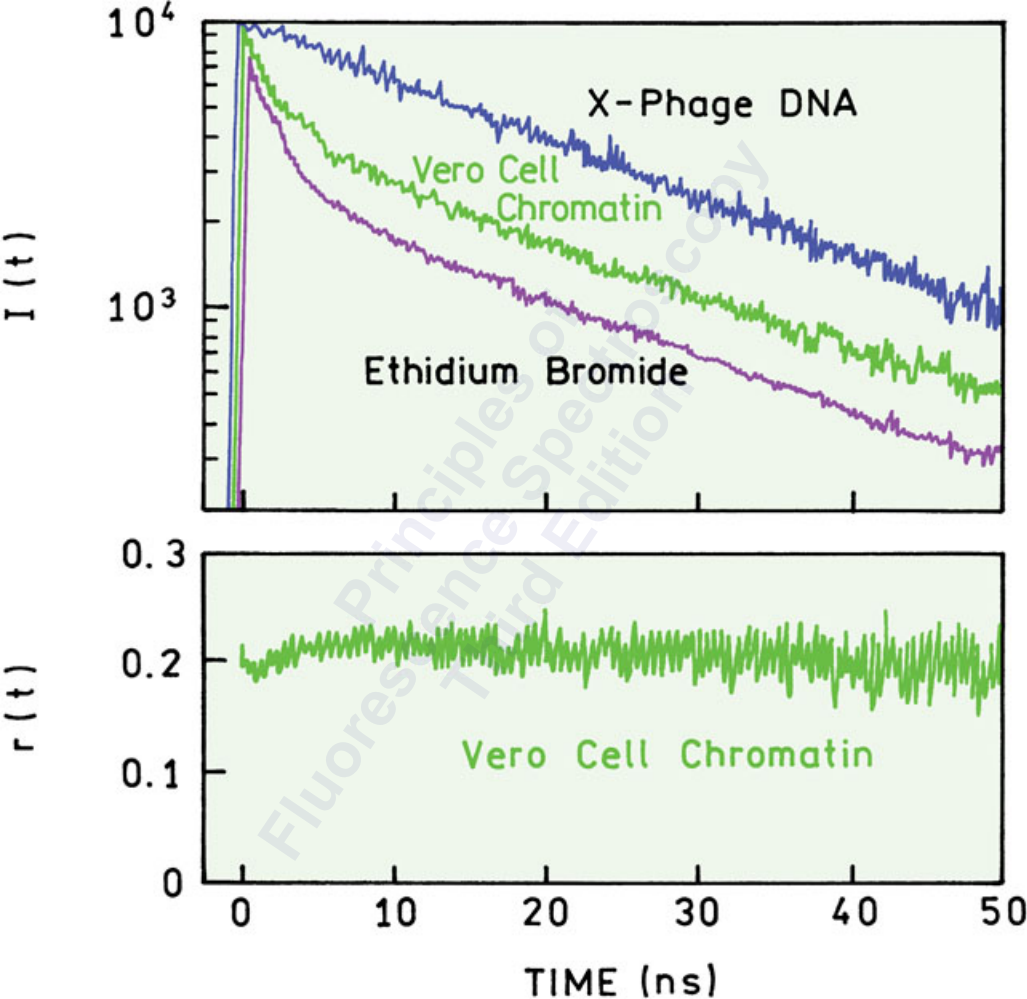
MODULATION RATIOS

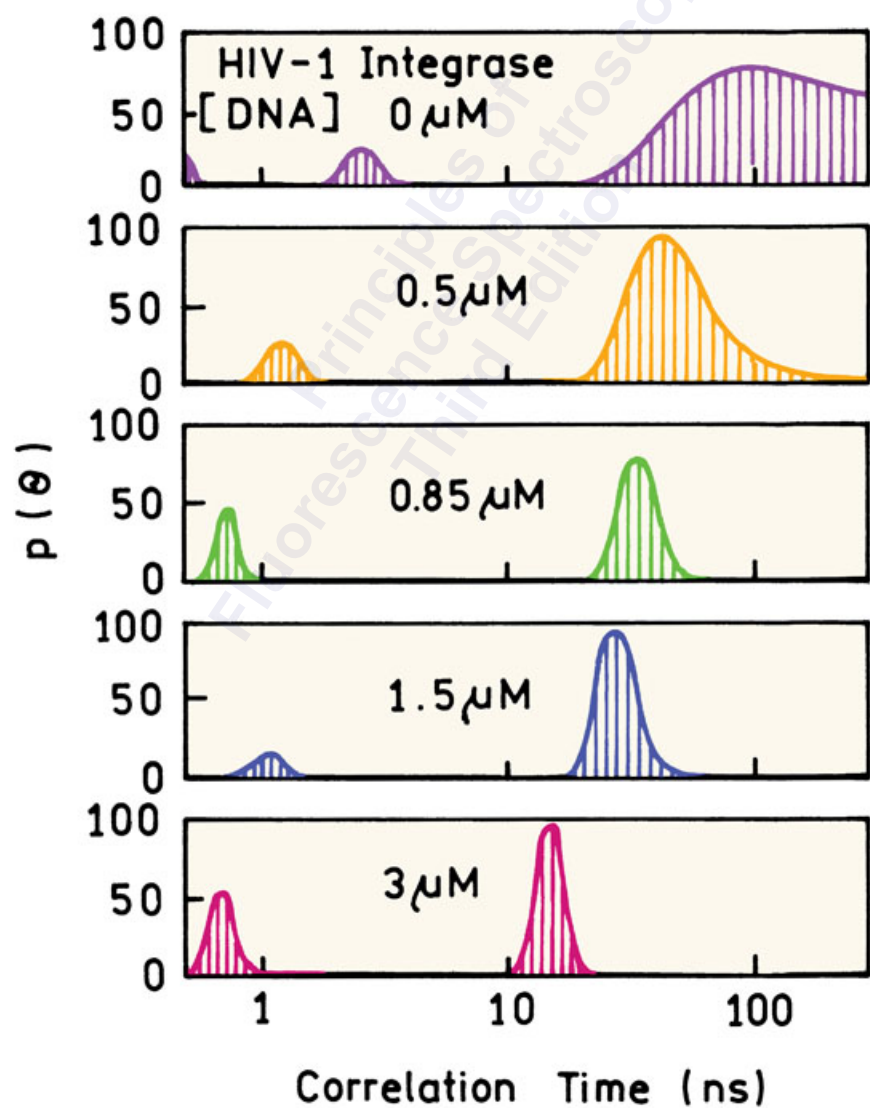
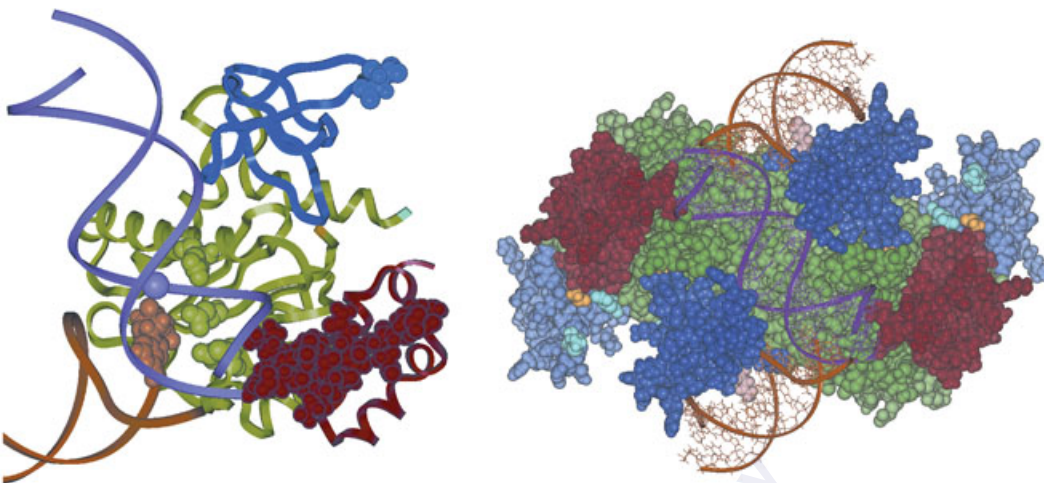
0.7  
0.6  
0.5  
0.4

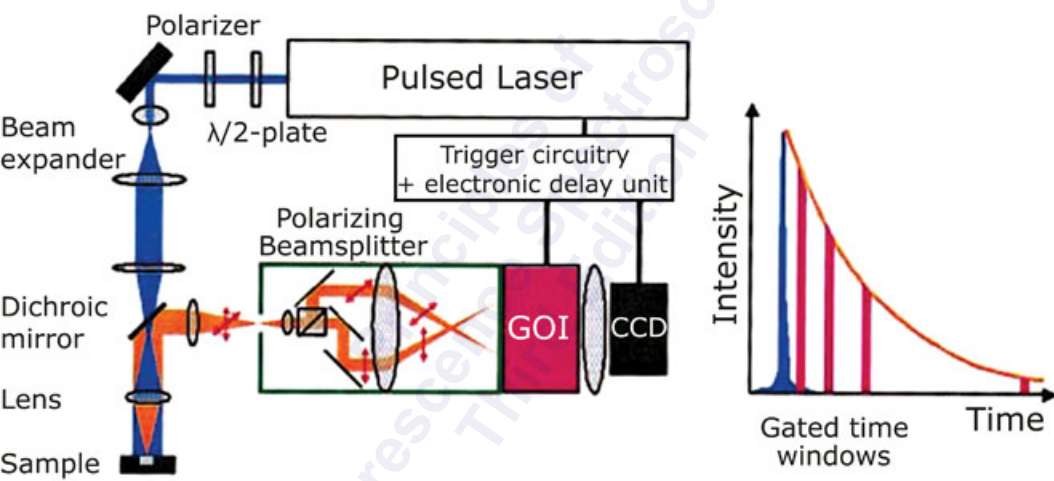
PHASE DIFFERENCES

12  
8  
4  
0

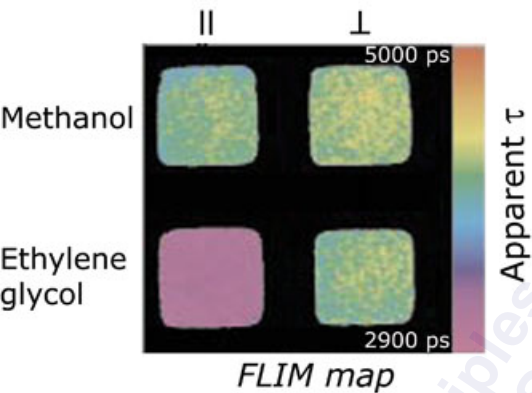
FREQUENCY (MHz)



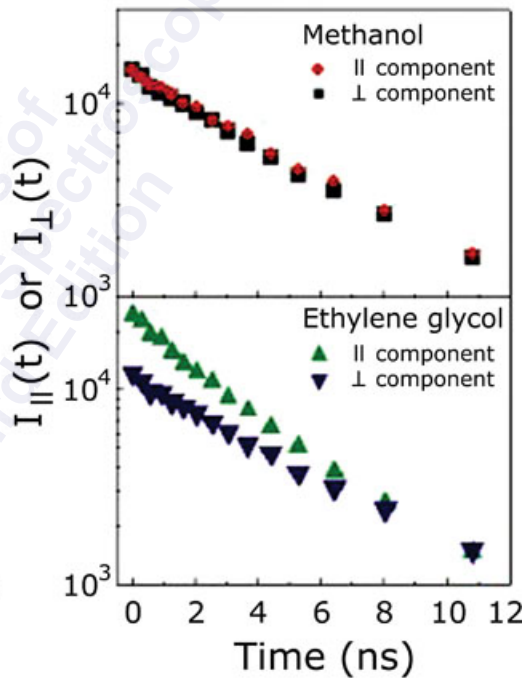
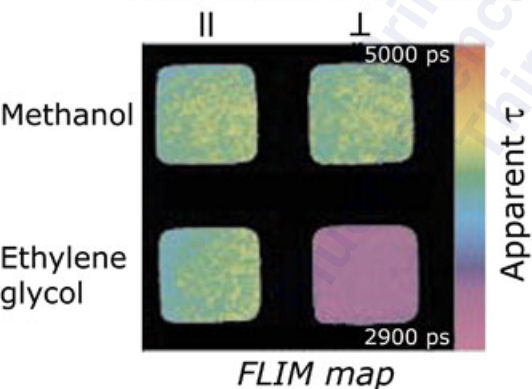


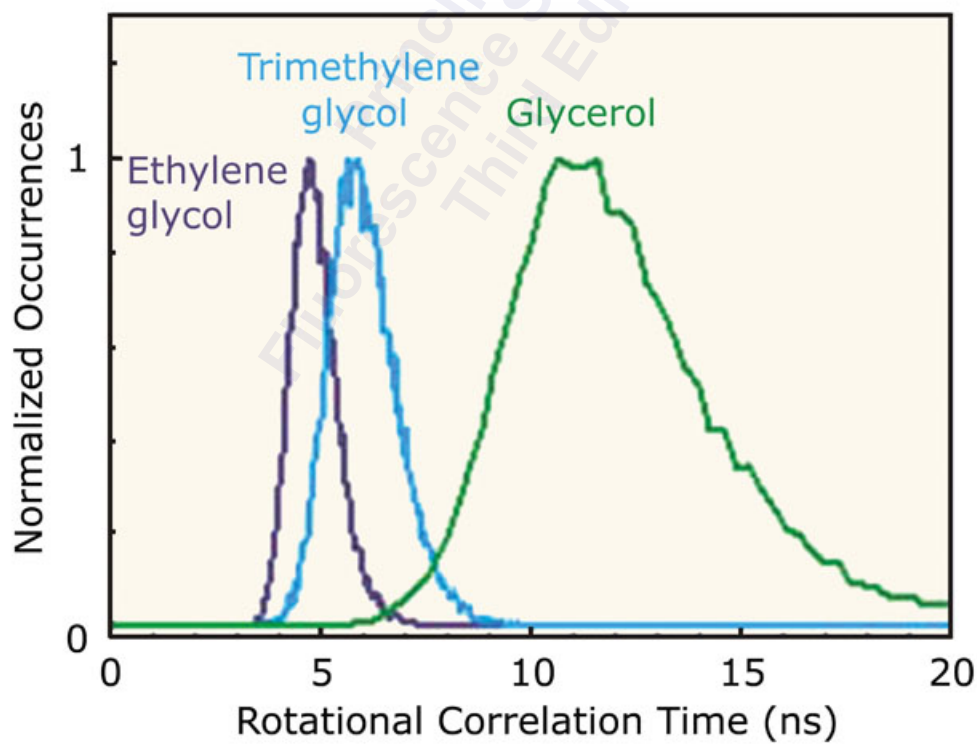
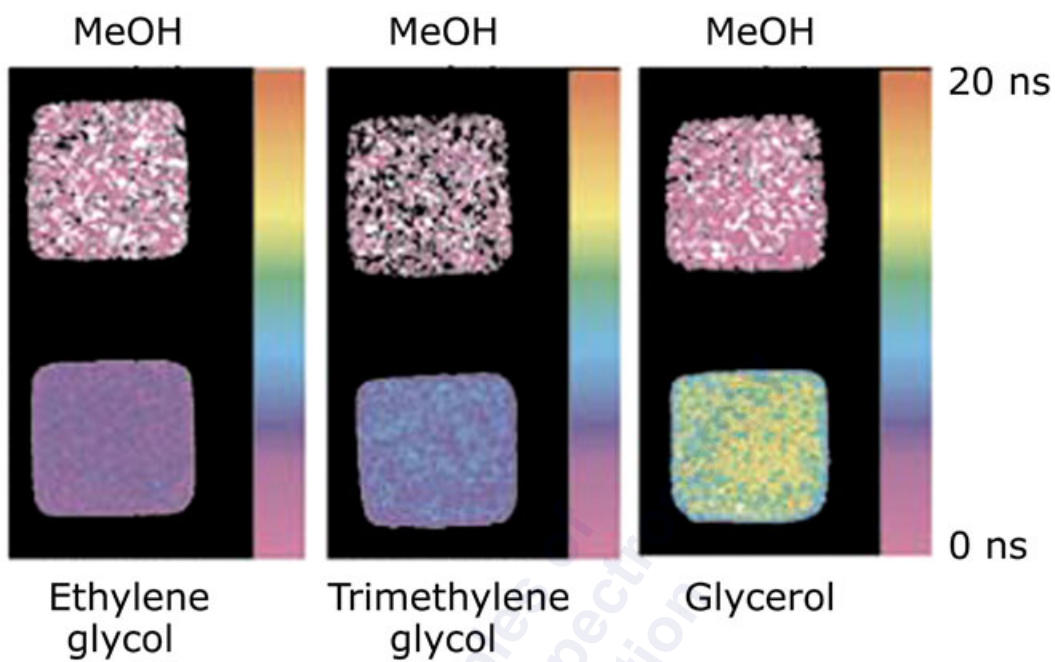


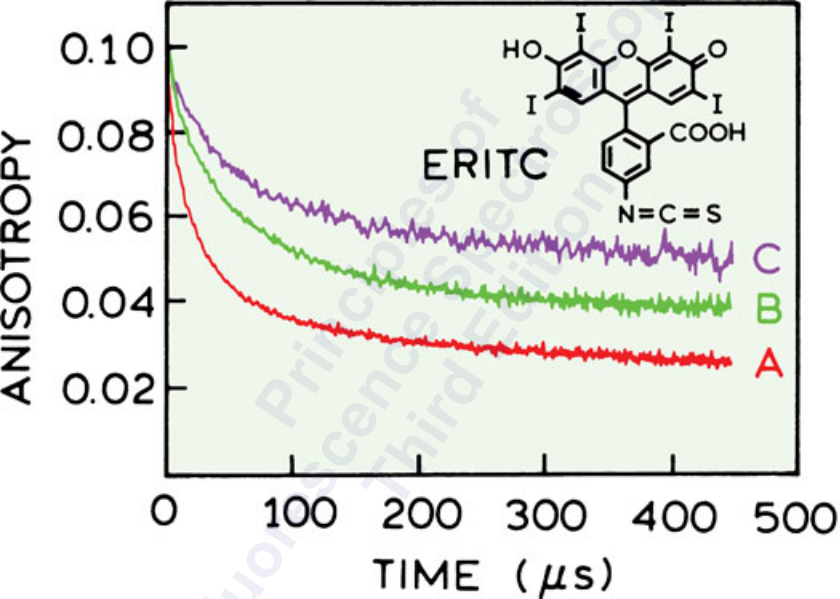
## Vertical excitation



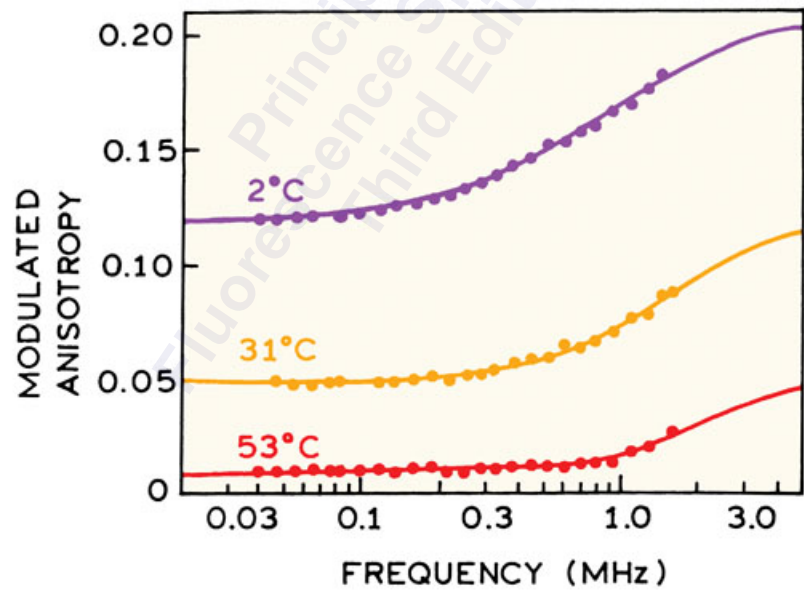
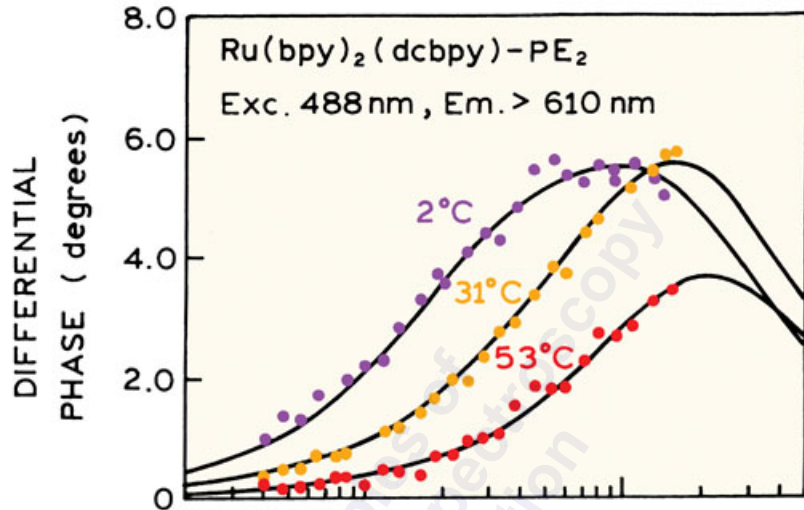
## Horizontal excitation





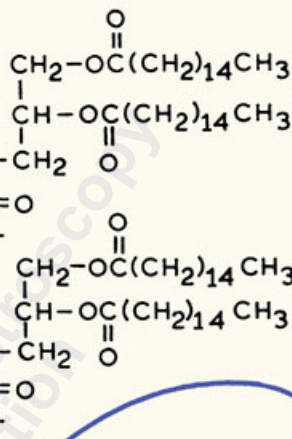
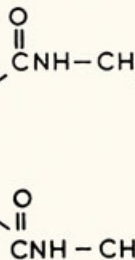
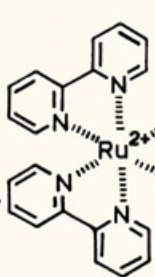






DIFFERENTIAL PHASE ( $\Delta\omega$ )

$\text{Ru}(\text{bpy})_2(\text{dcbpy})-\text{PE}_2$



6.0

4.0

2.0

0

DPPG,  $2^\circ\text{C}$

$\theta_1 = 1761 \text{ ns}$

$r_0 g_1 = 0.145$

$\theta_2 = 133 \text{ ns}$

$r_0 g_2 = 0.062$

FREQUENCY (MHz)

0.01

0.03

0.1

0.3

1.0

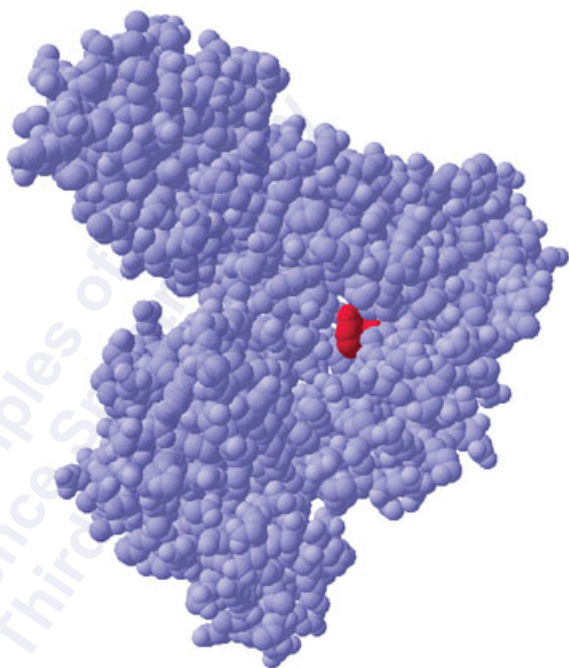
3.0



Trp

$\tau = 3\text{ns}$

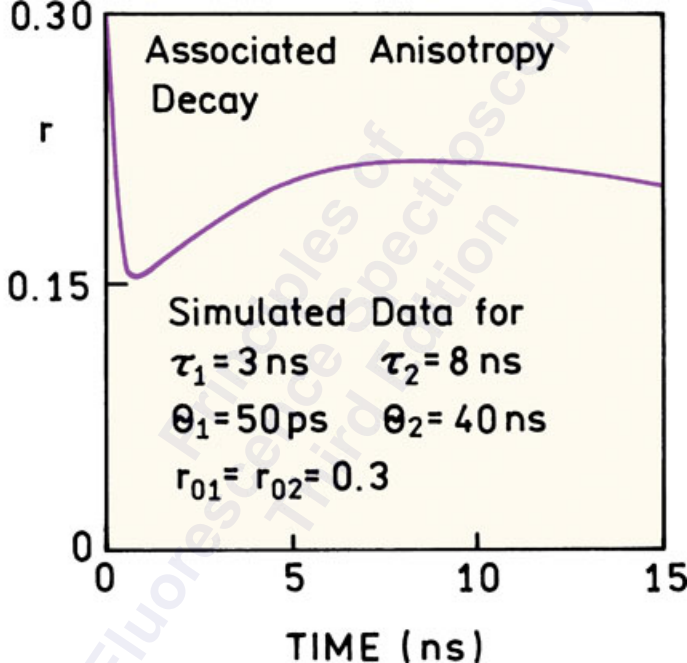
$\theta = 50\text{ps}$

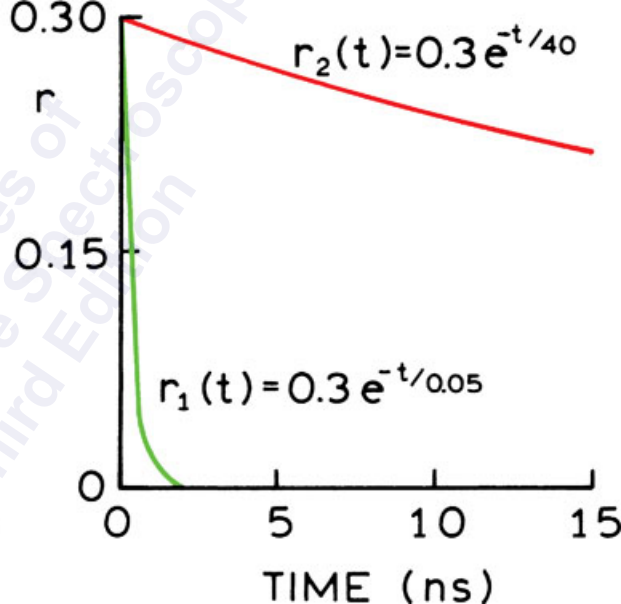
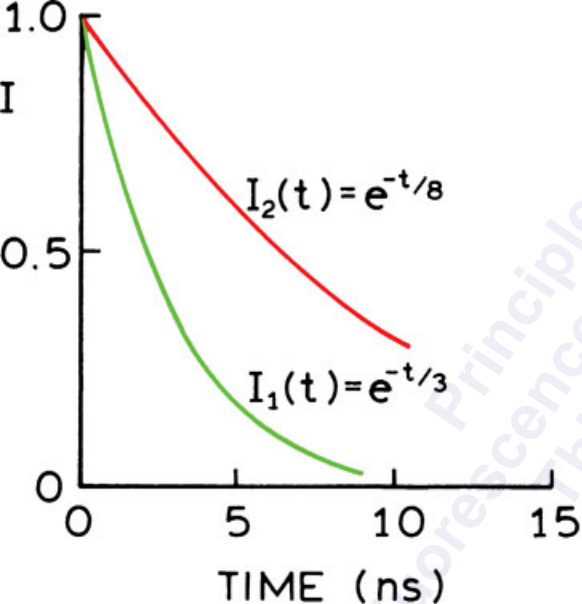


HSA

$\tau = 8\text{ns}$

$\theta = 40\text{ns}$





ANISOTROPY

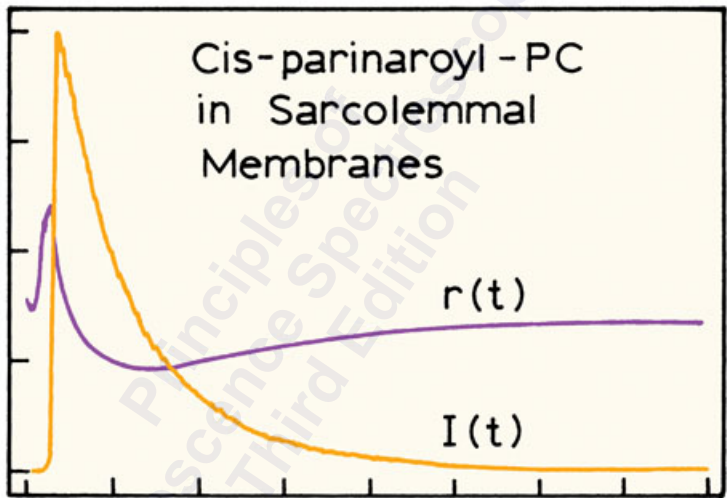
Cis-parinaroyl - PC  
in Sarcolemmal  
Membranes

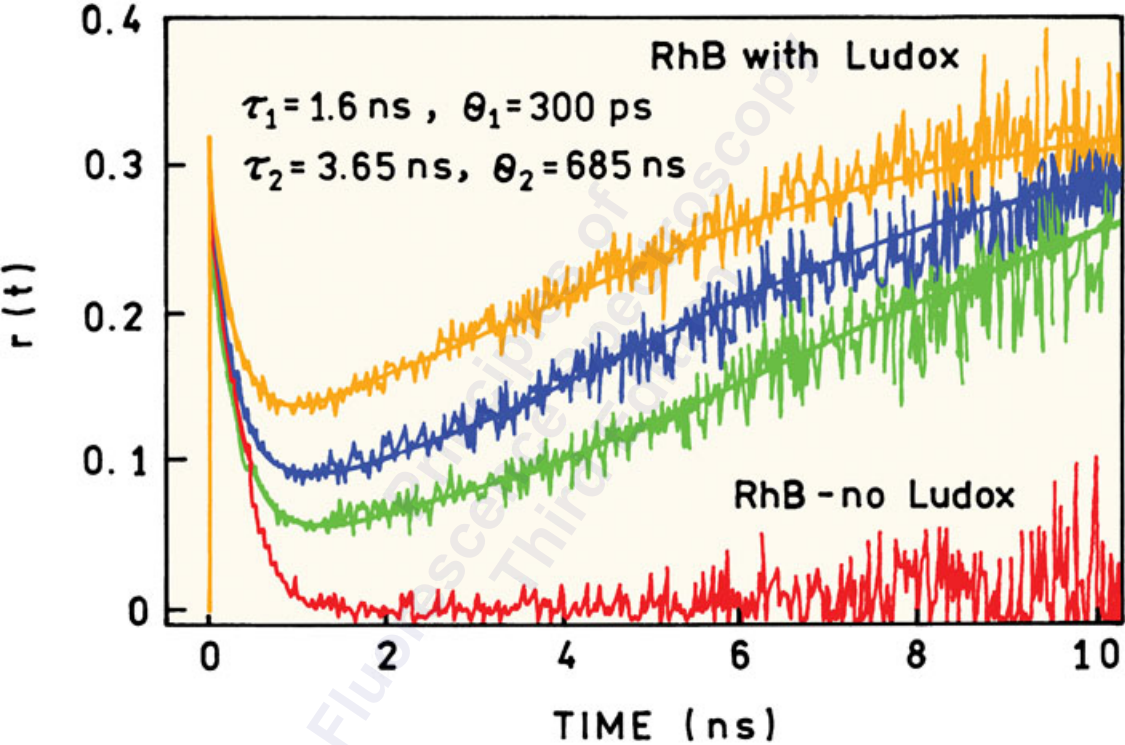
$r(t)$

$I(t)$

0 20 40 60 80

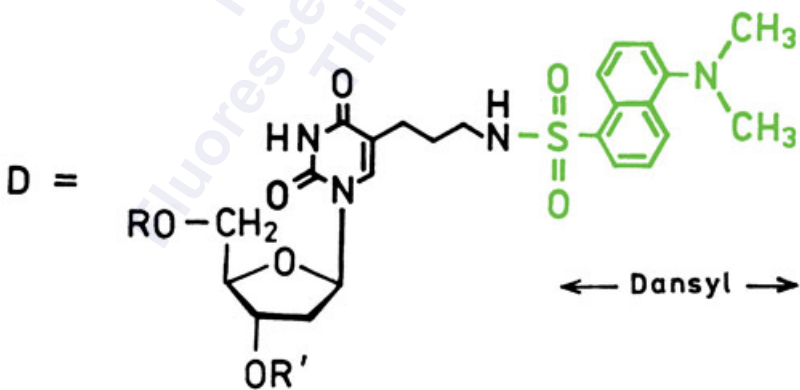
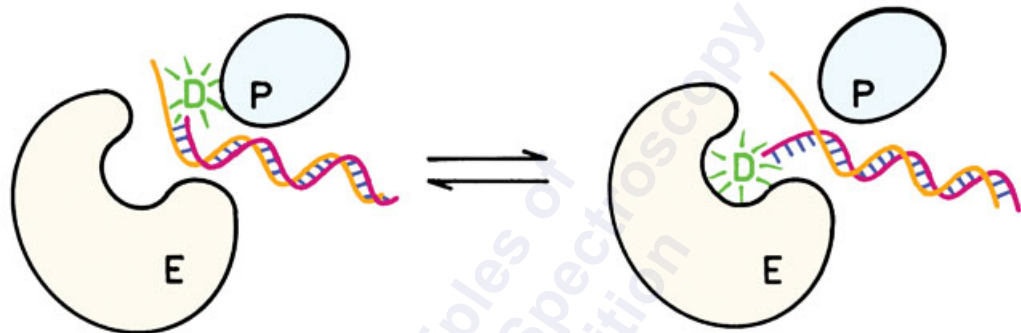
TIME (ns)



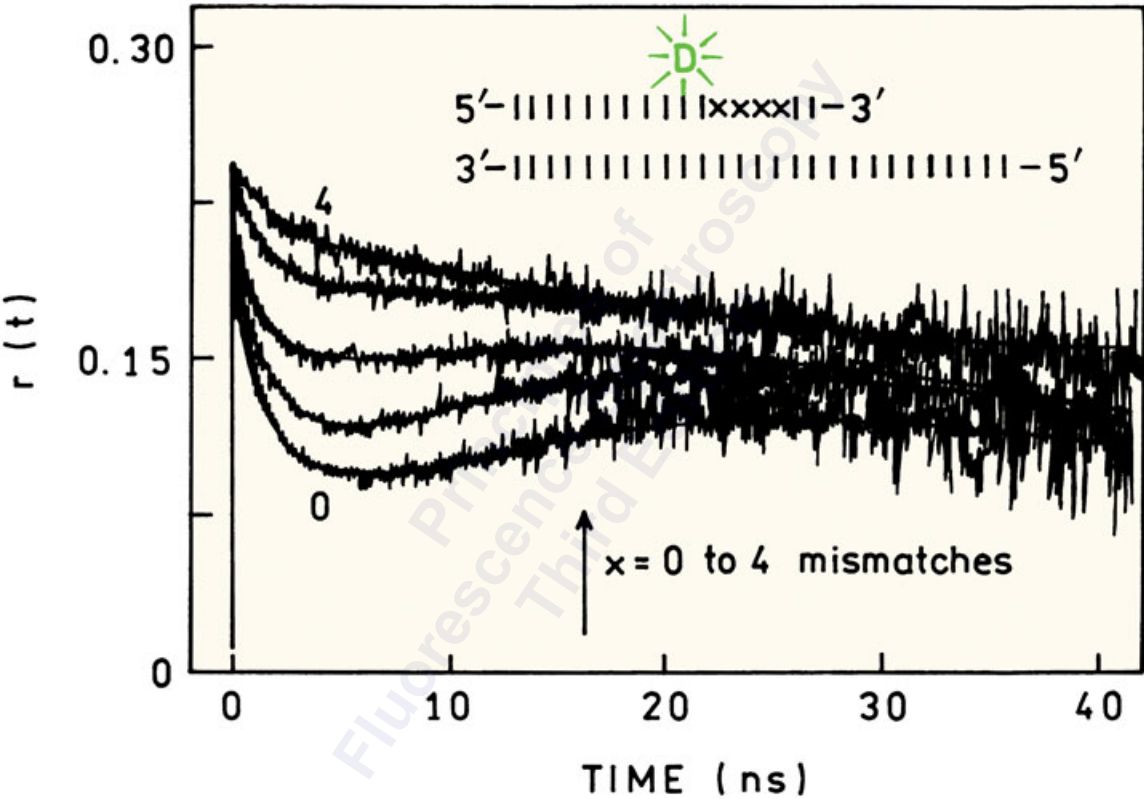


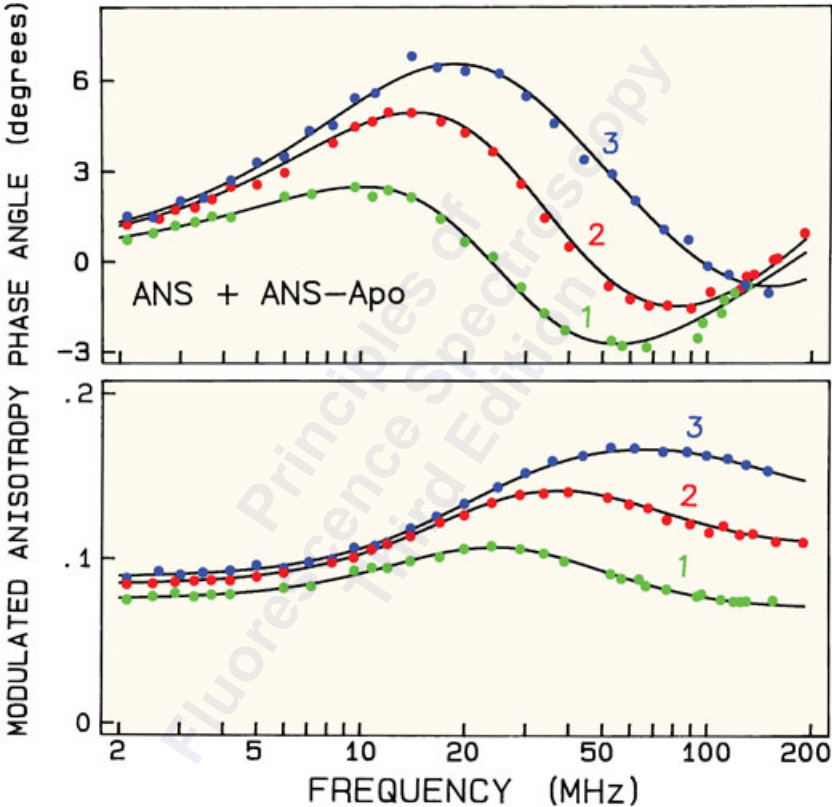
Polymerase mode  
(replicative complex)

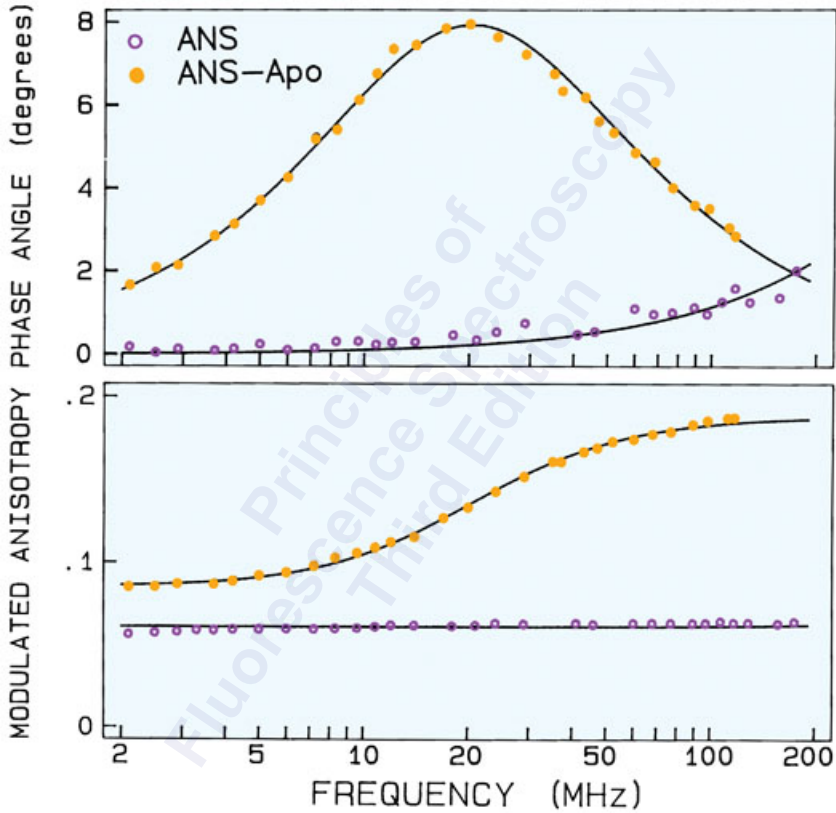
Exonuclease mode  
(editing complex)

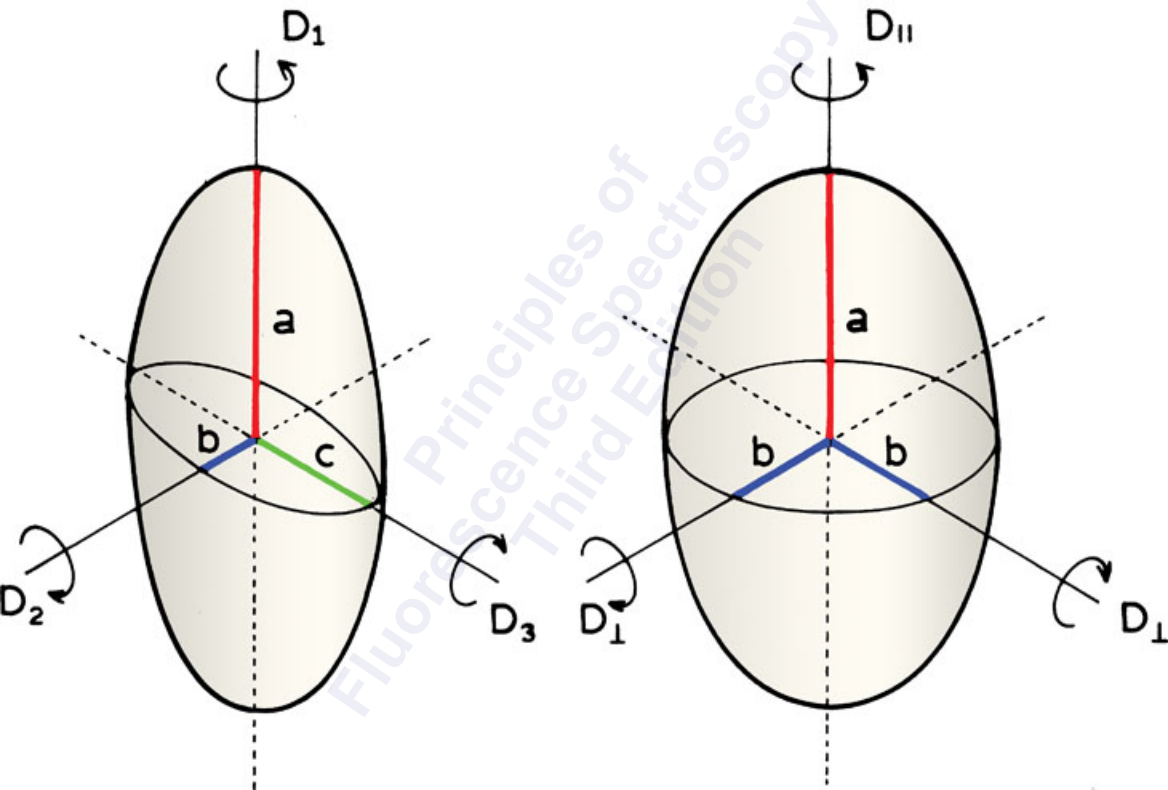






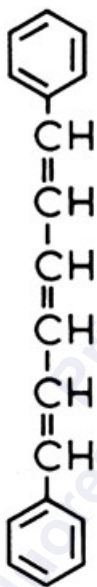
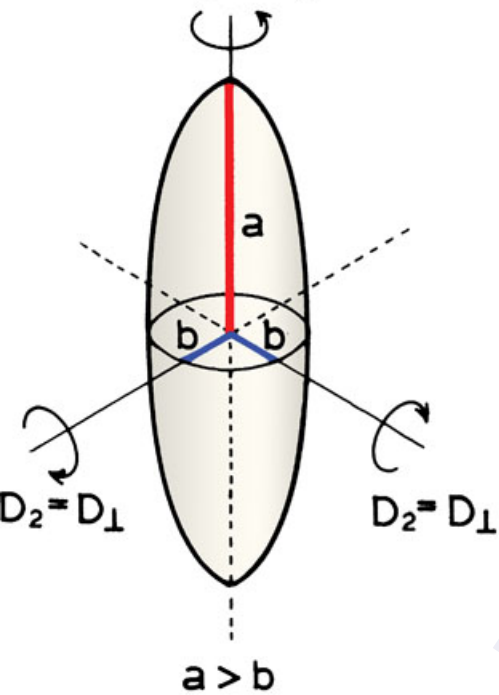






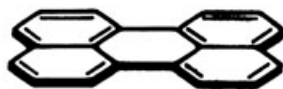
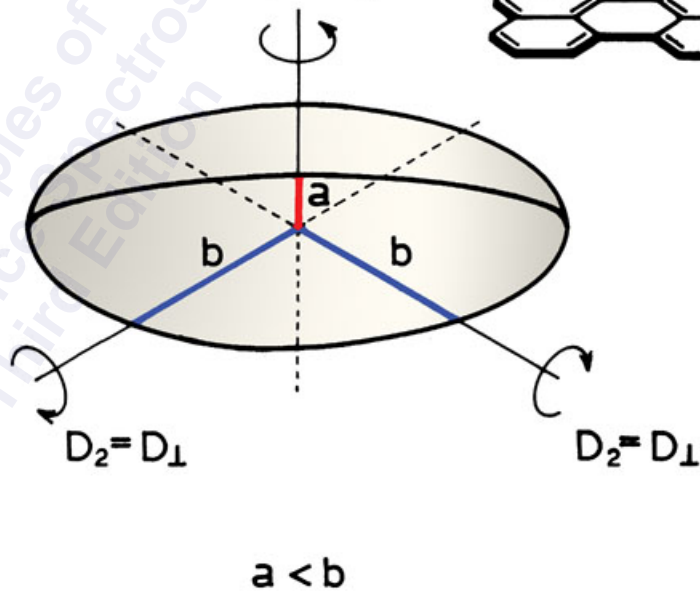
Prolate

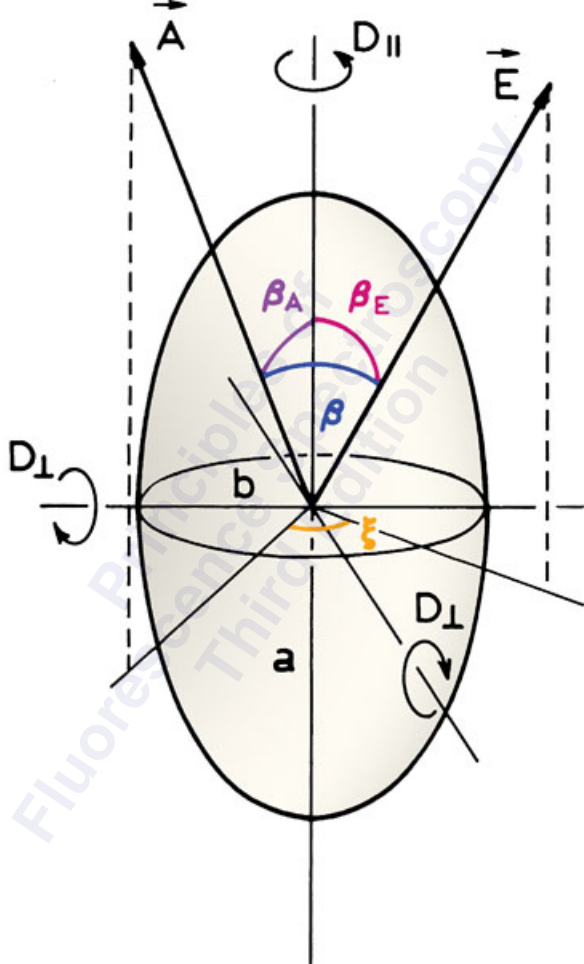
$$D_1 = D_{||}$$

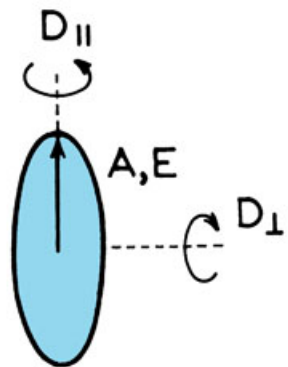


Oblate

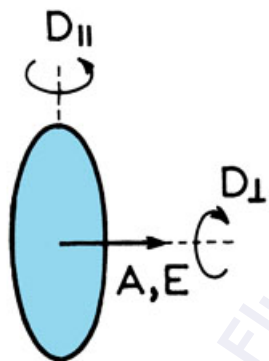
$$D_1 = D_{||}$$



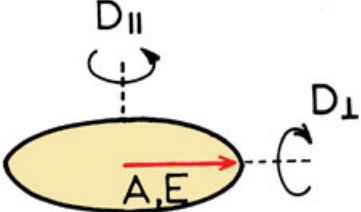




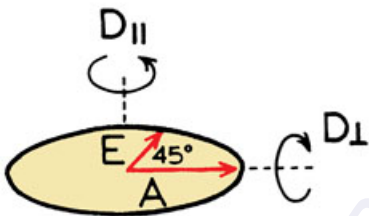
$$r(t) = 0.4 \exp(-6 D_{\perp} t)$$



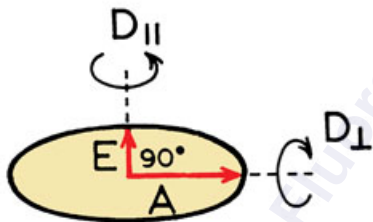
$$r(t) = 0.3 \exp(-4 D_{||} t - 2 D_{\perp} t) + 0.1 \exp(-6 D_{\perp} t)$$



$$r(t) = 0.3 \exp(-4 D_{||} t - 2 D_{\perp} t) + 0.1 \exp(-6 D_{\perp} t)$$

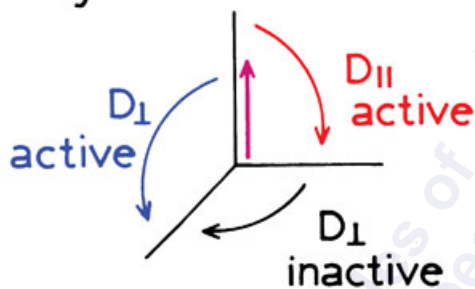
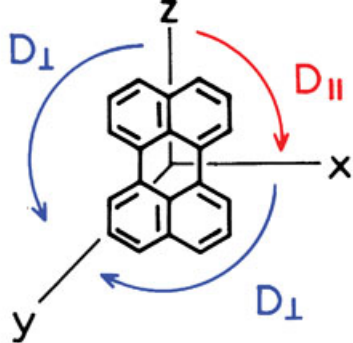


$$r(t) = 0.1 \exp(-6 D_{\perp} t)$$



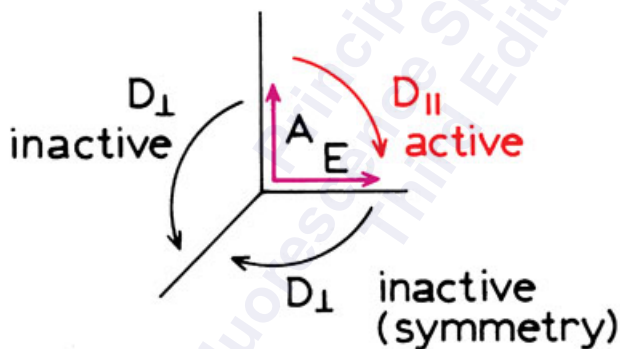
$$r(t) = -0.3 \exp(-4 D_{||} t - 2 D_{\perp} t) + 0.1 \exp(-6 D_{\perp} t)$$





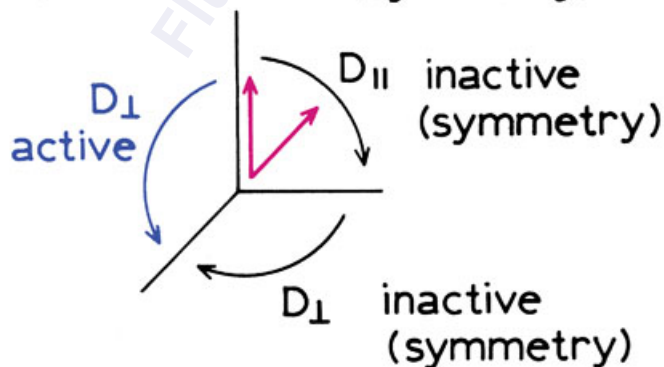
$$r_o = 0.4$$

$$\beta = 0^\circ$$



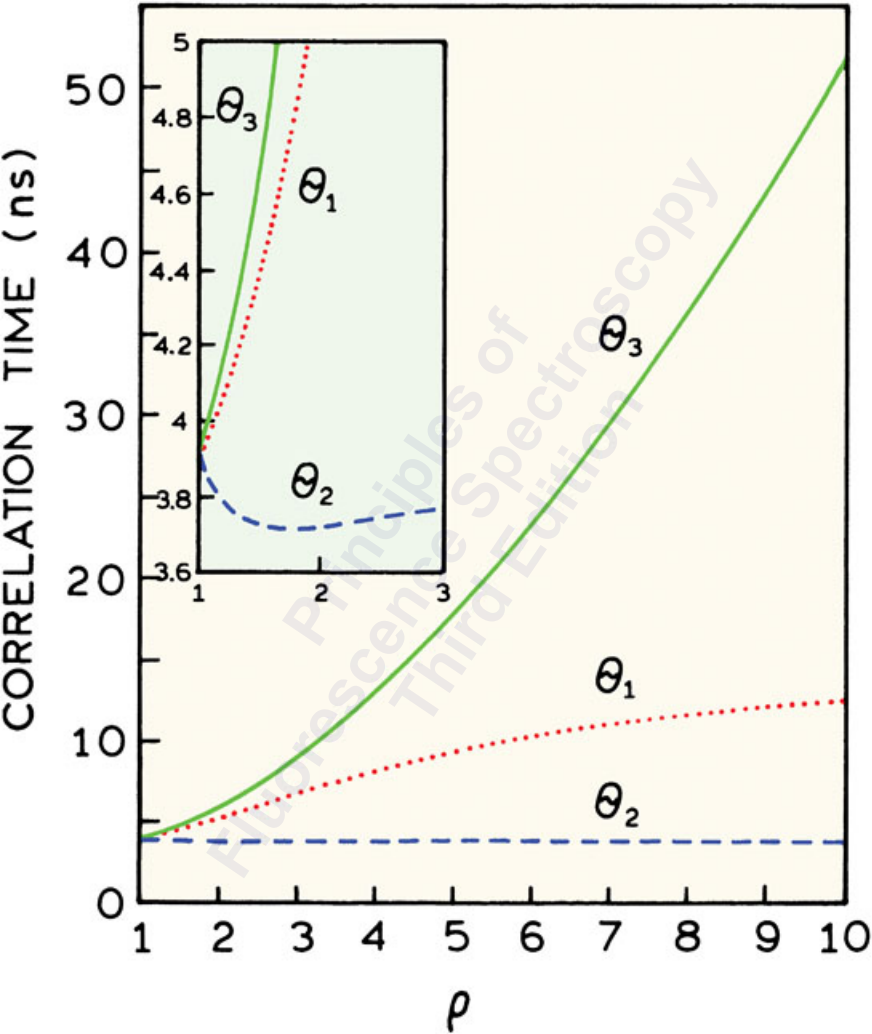
$$r_o = -0.2$$

$$\beta = 90^\circ$$

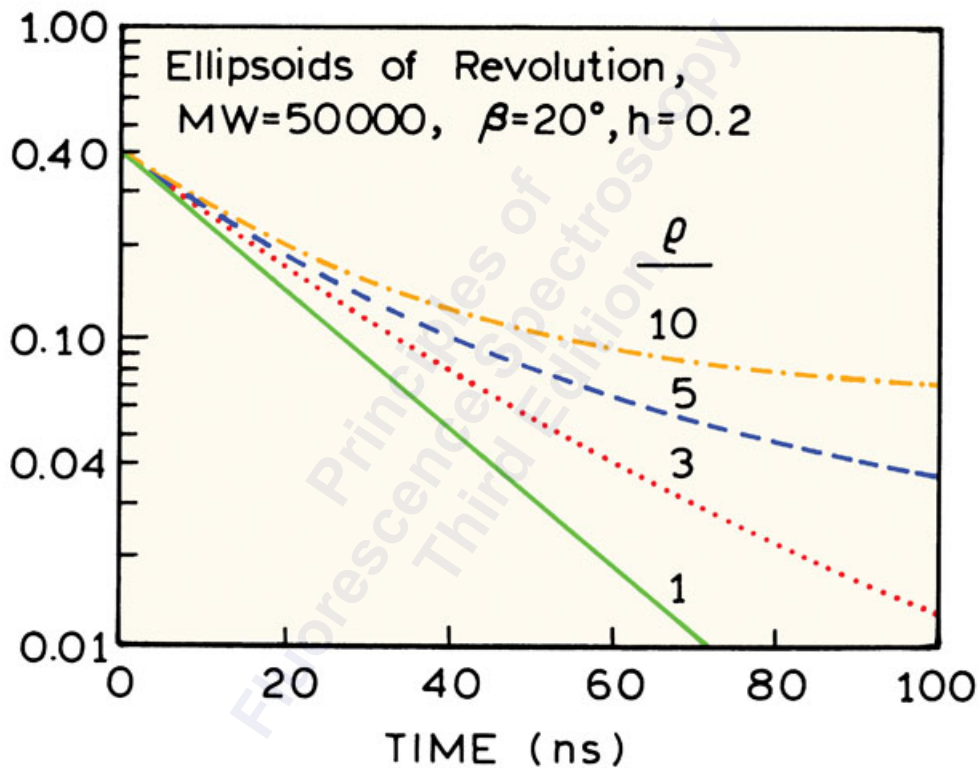


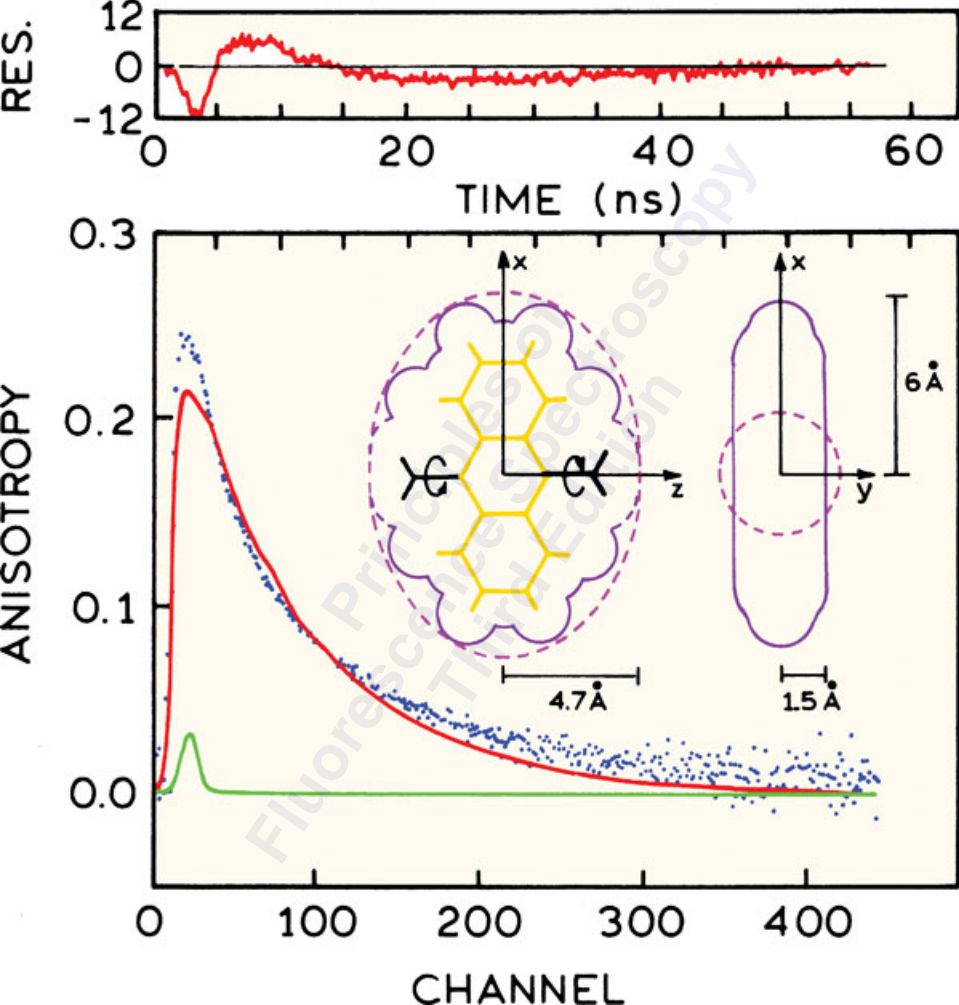
$$r_o = 0.1$$

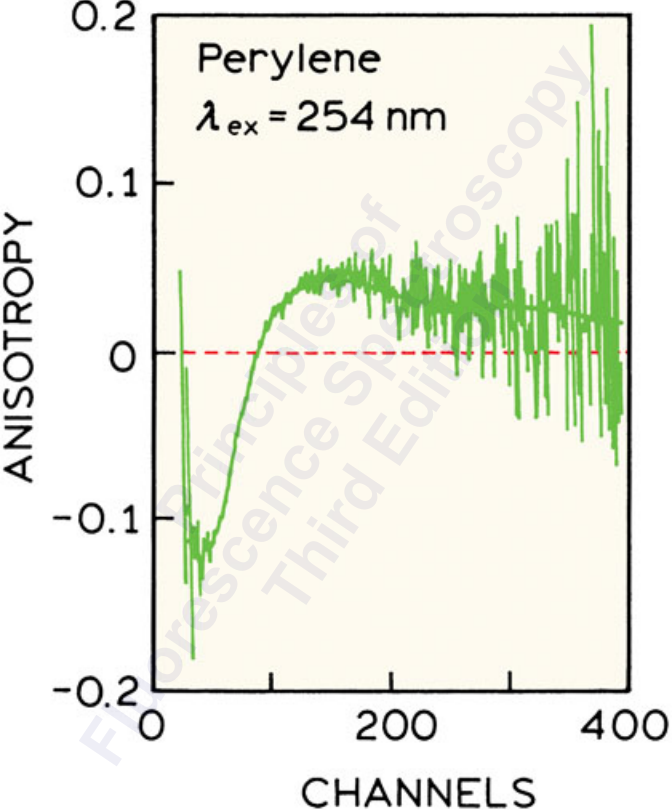
$$\beta = 45^\circ$$

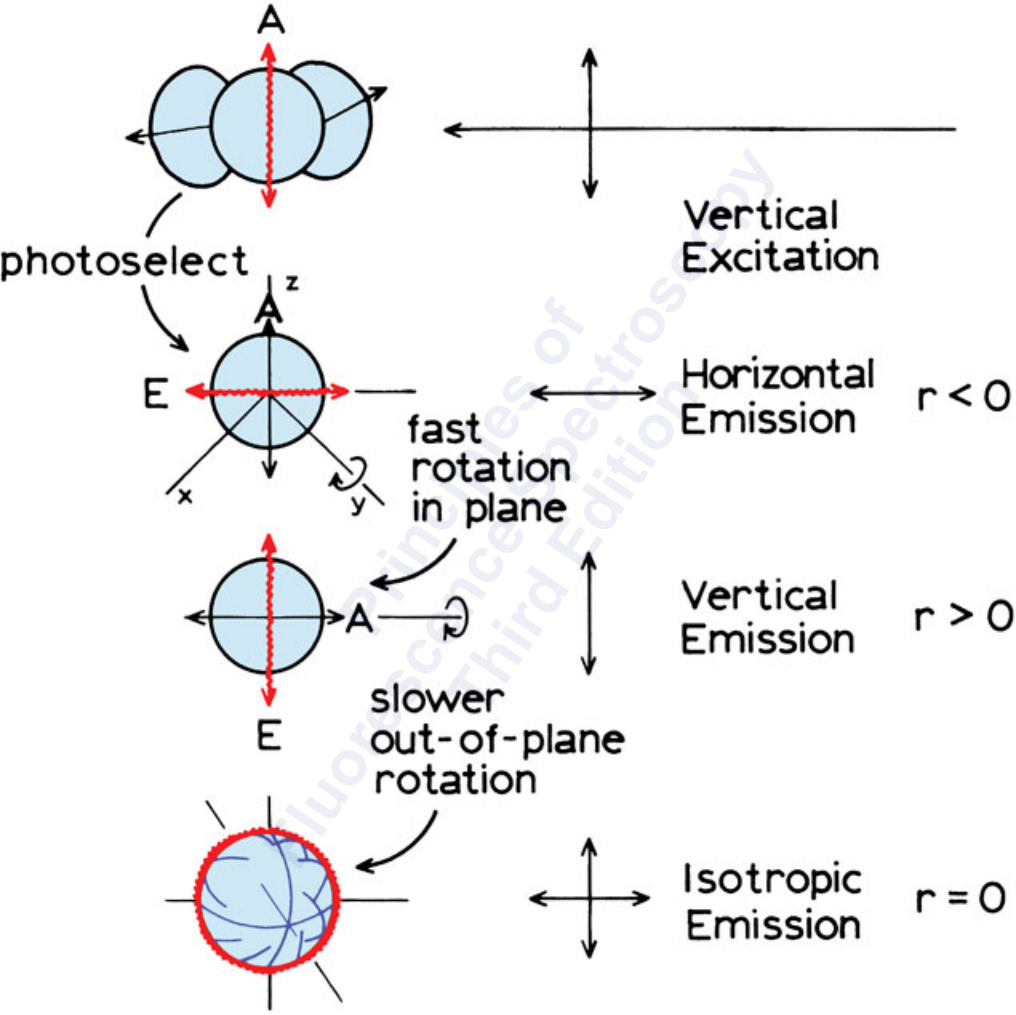


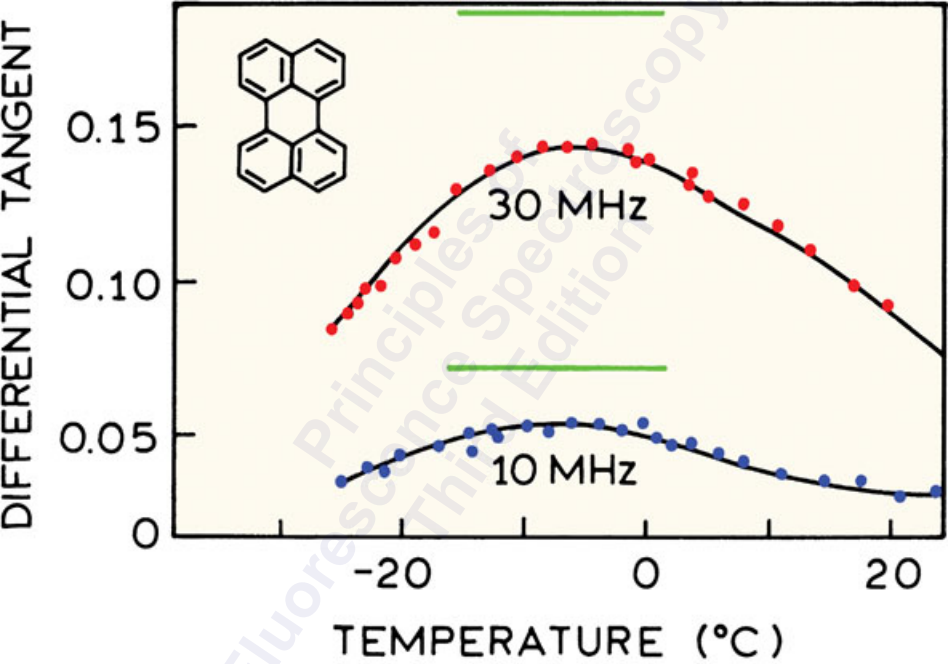
ANISOTROPY

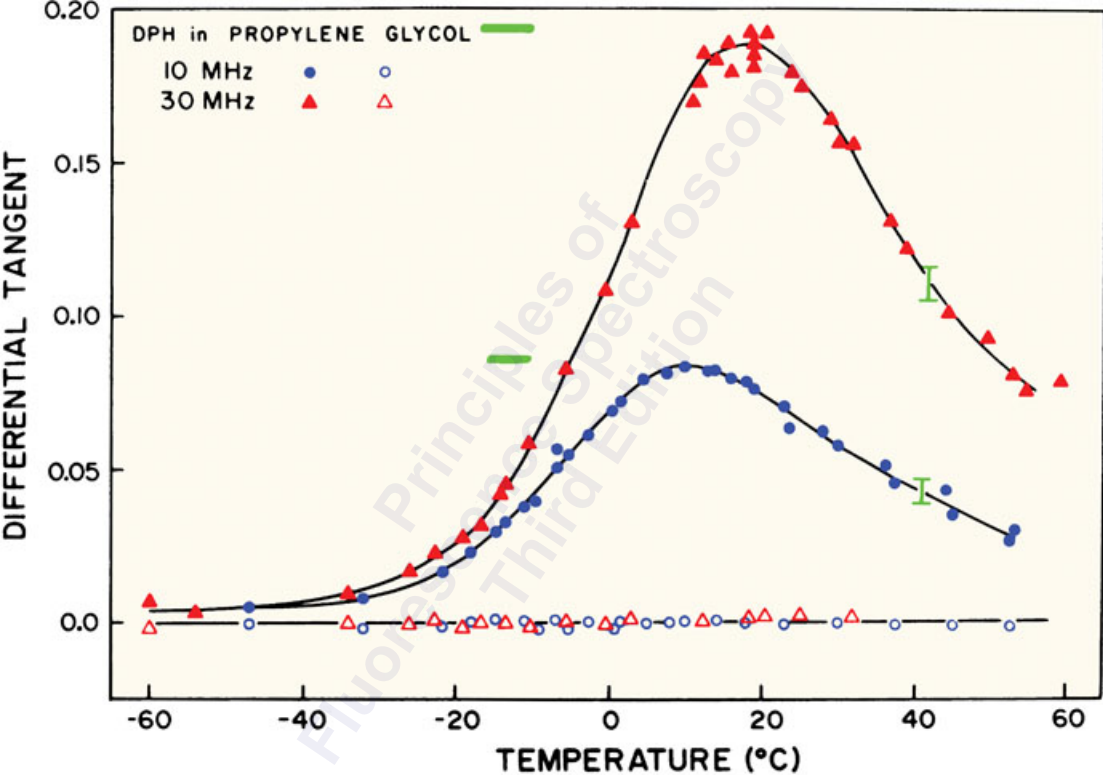










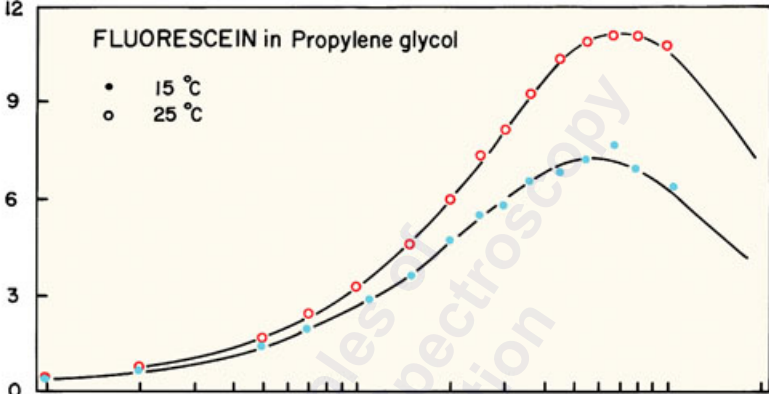




DIFFERENTIAL POLARIZED  
PHASE ANGLE (degrees)

FLUORESCCEIN in Propylene glycol

- 15 °C
- 25 °C

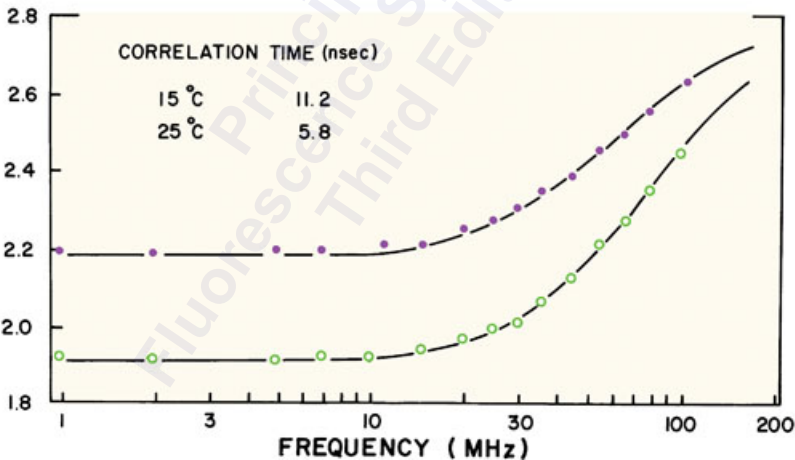


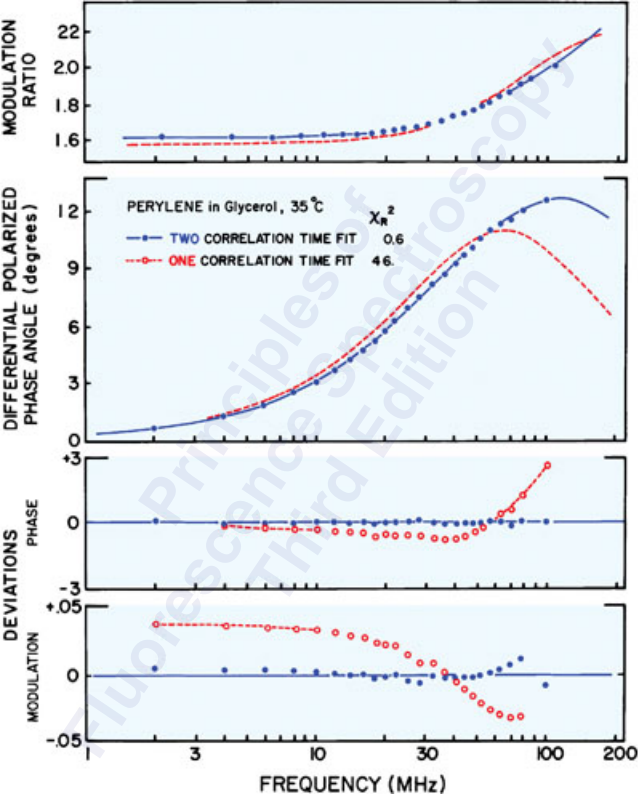
RATIO OF POLARIZED  
MODULATED AMPLITUDES

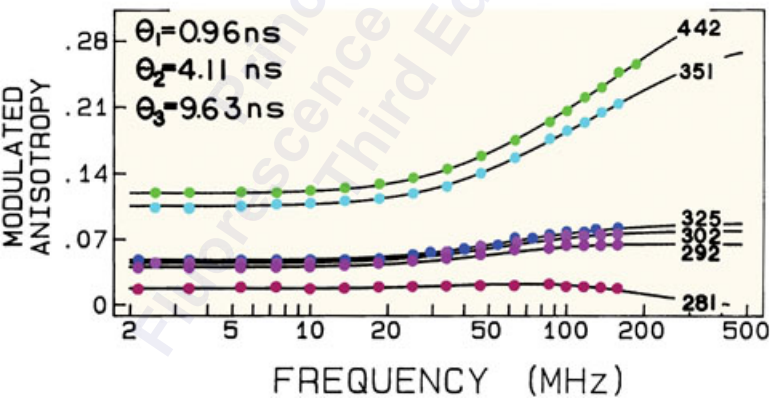
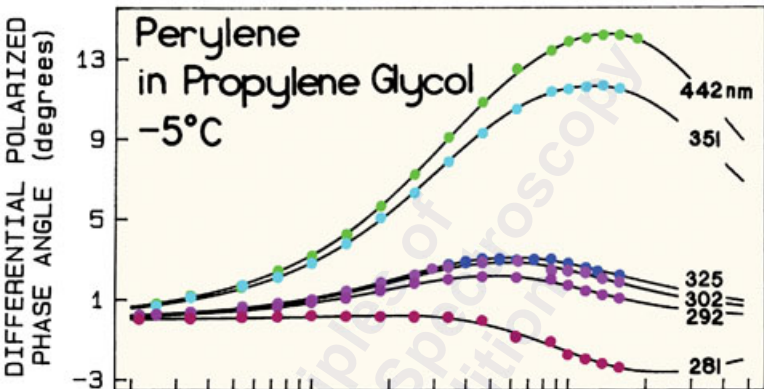
CORRELATION TIME (nsec)

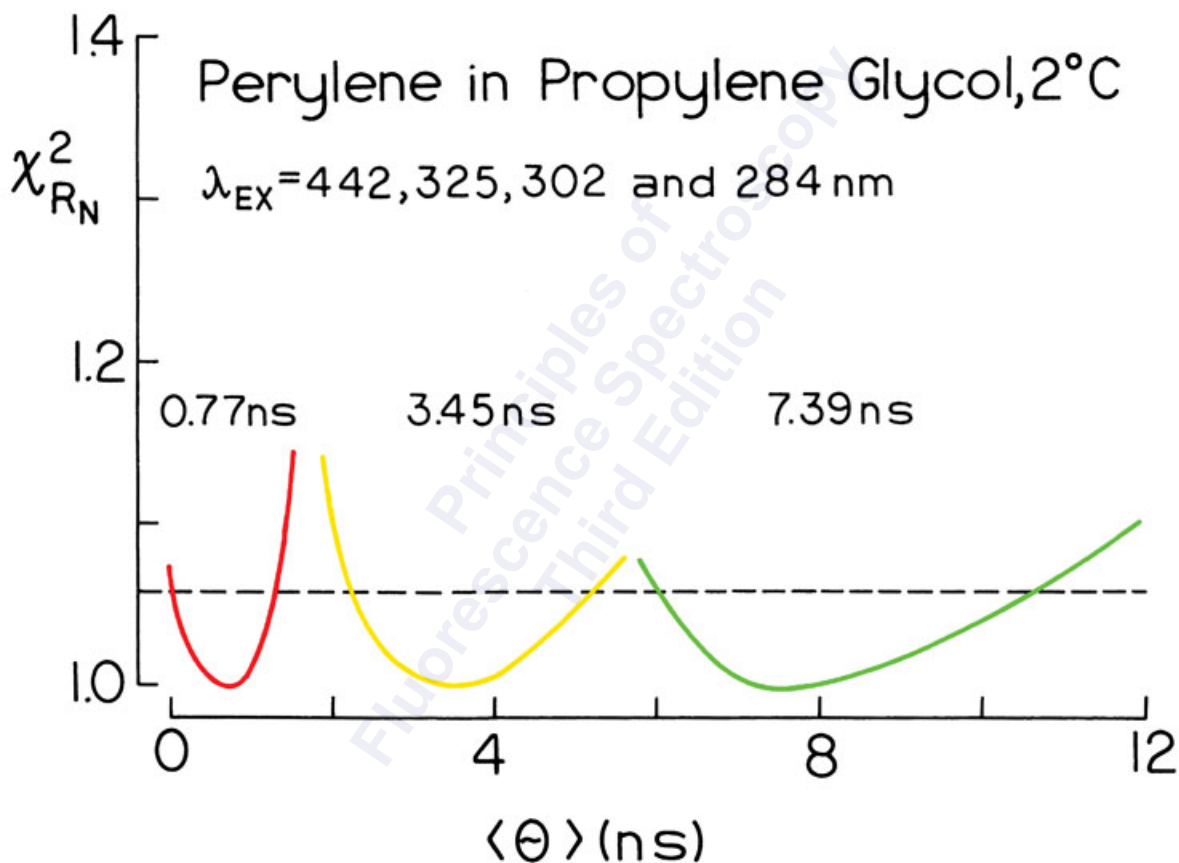
- 15 °C
- 25 °C

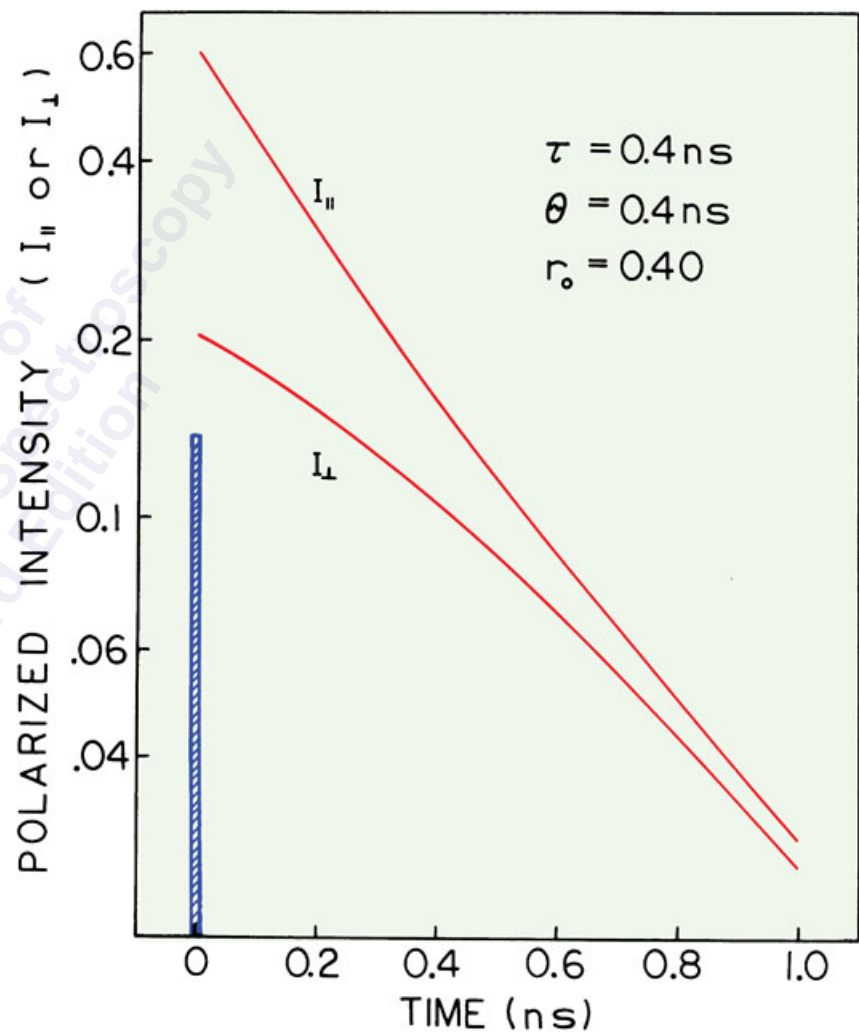
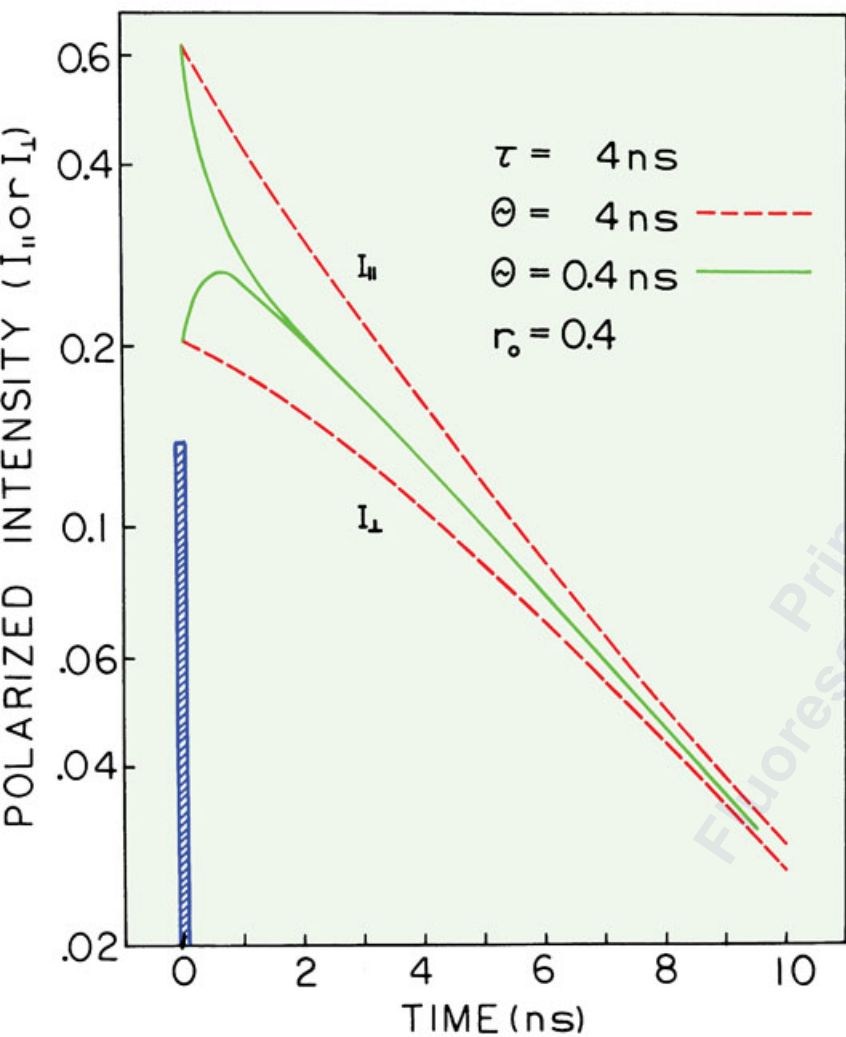
11.2  
5.8

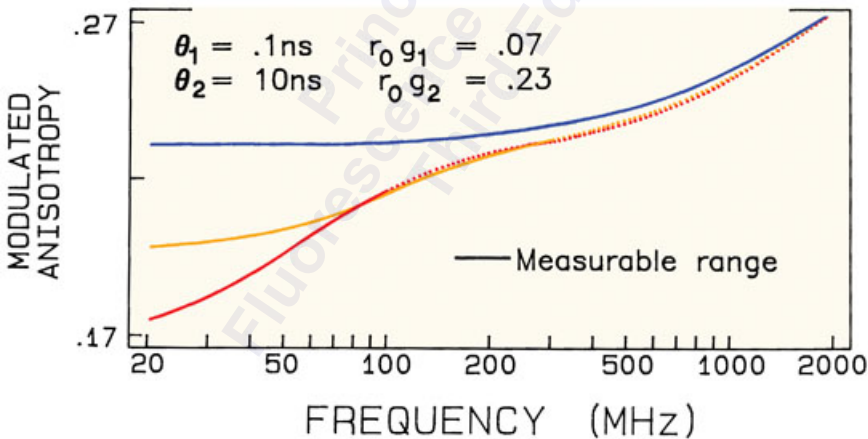
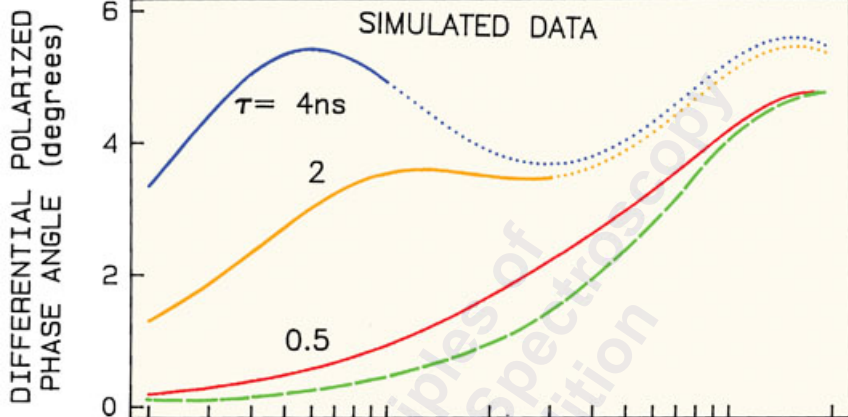




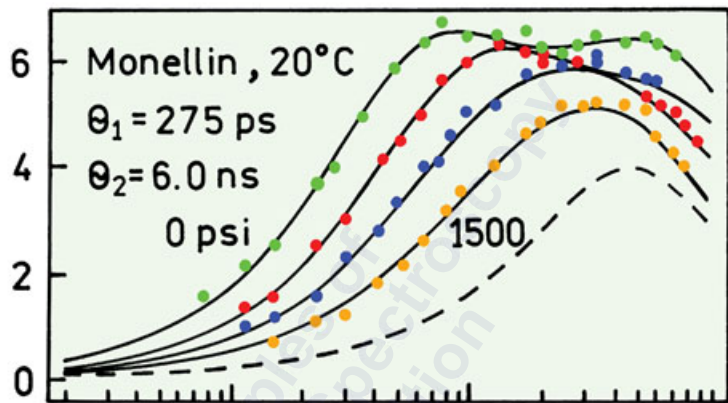




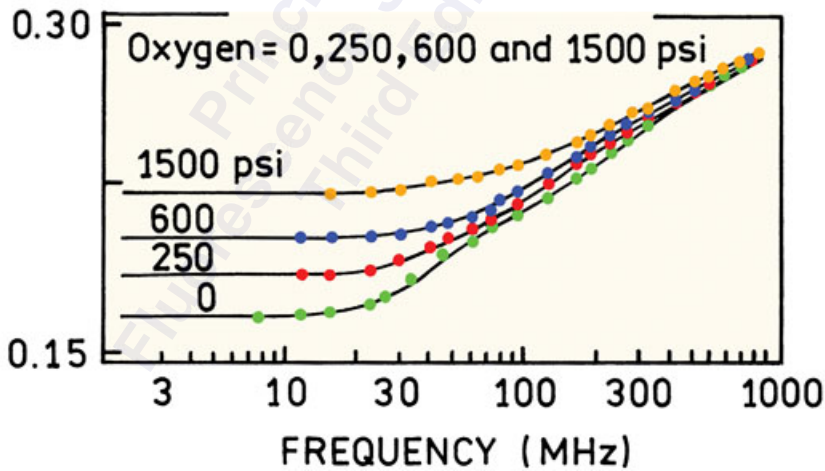


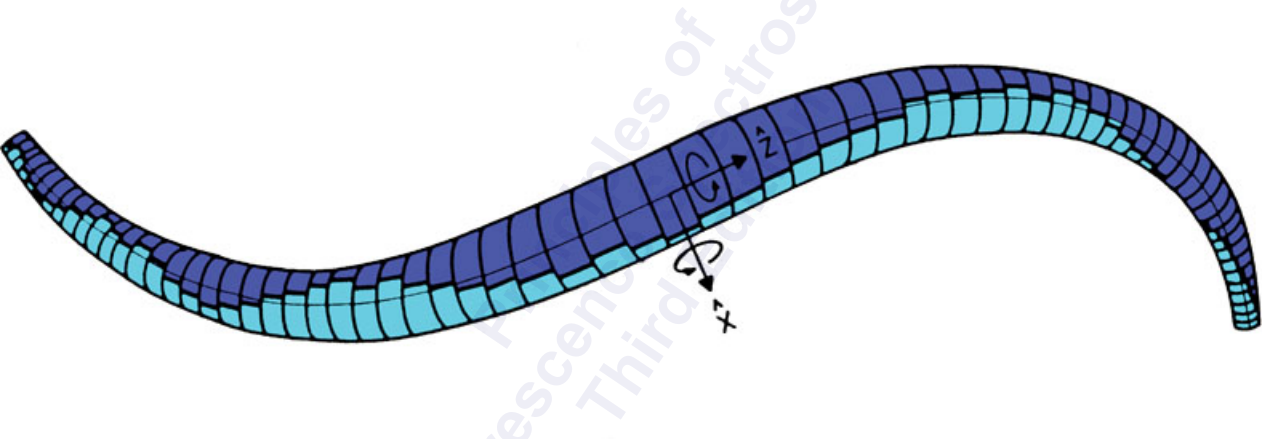


DIFFERENTIAL POLARIZED  
PHASE ANGLE (deg)



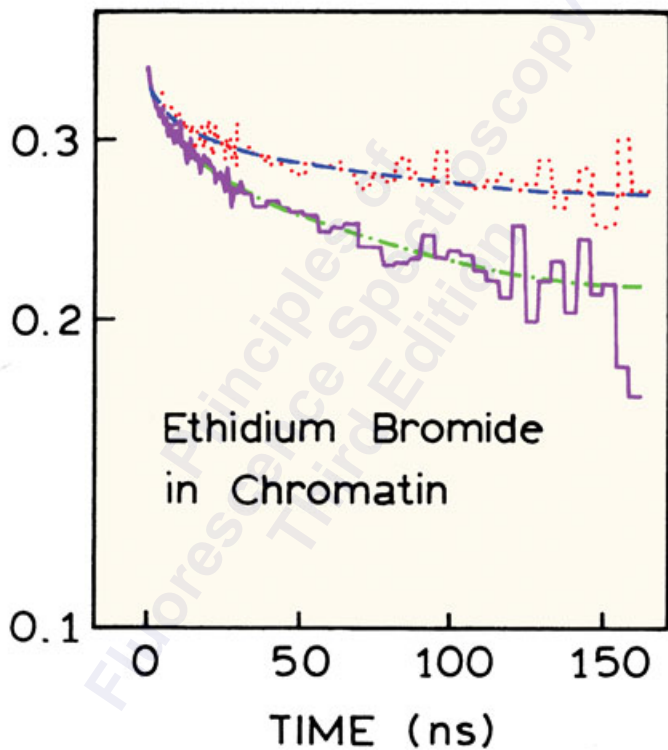
MODULATED  
ANISOTROPY

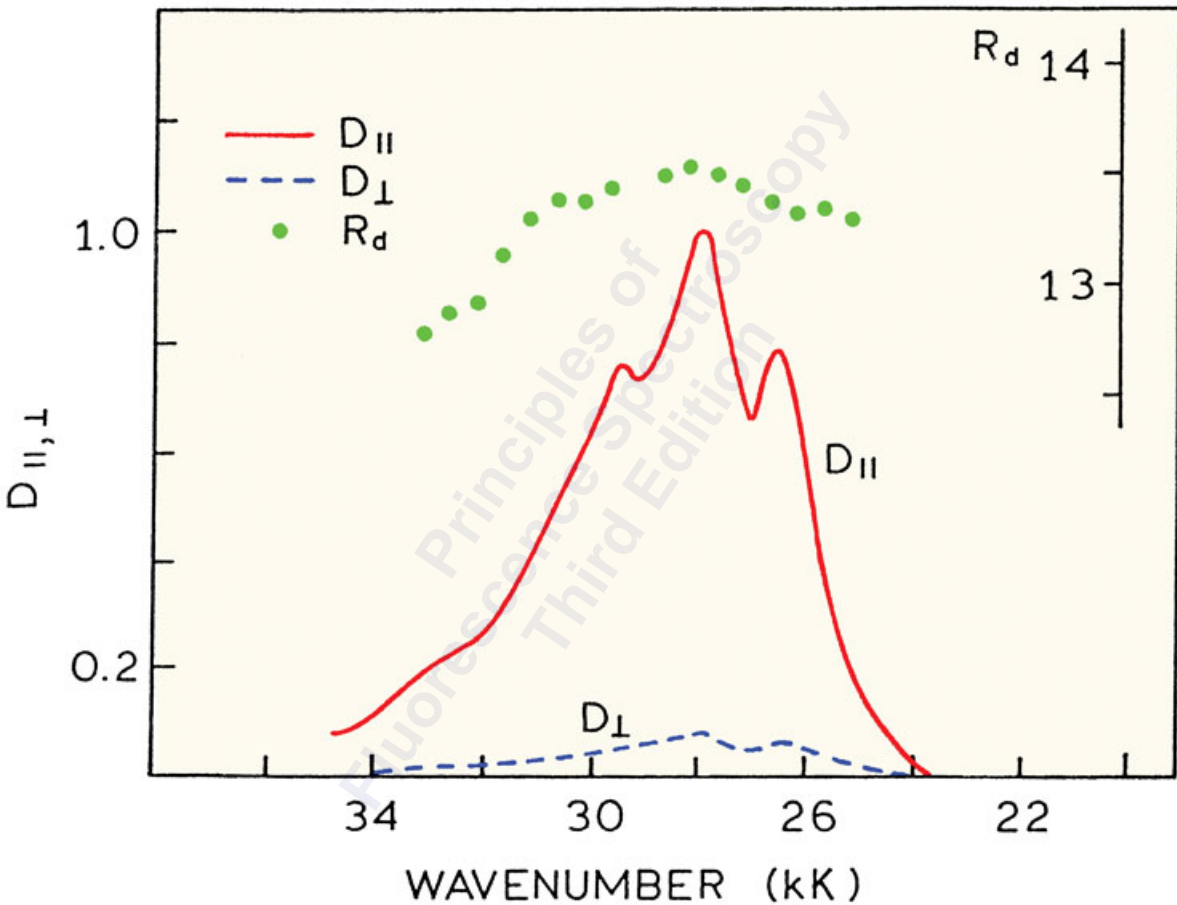


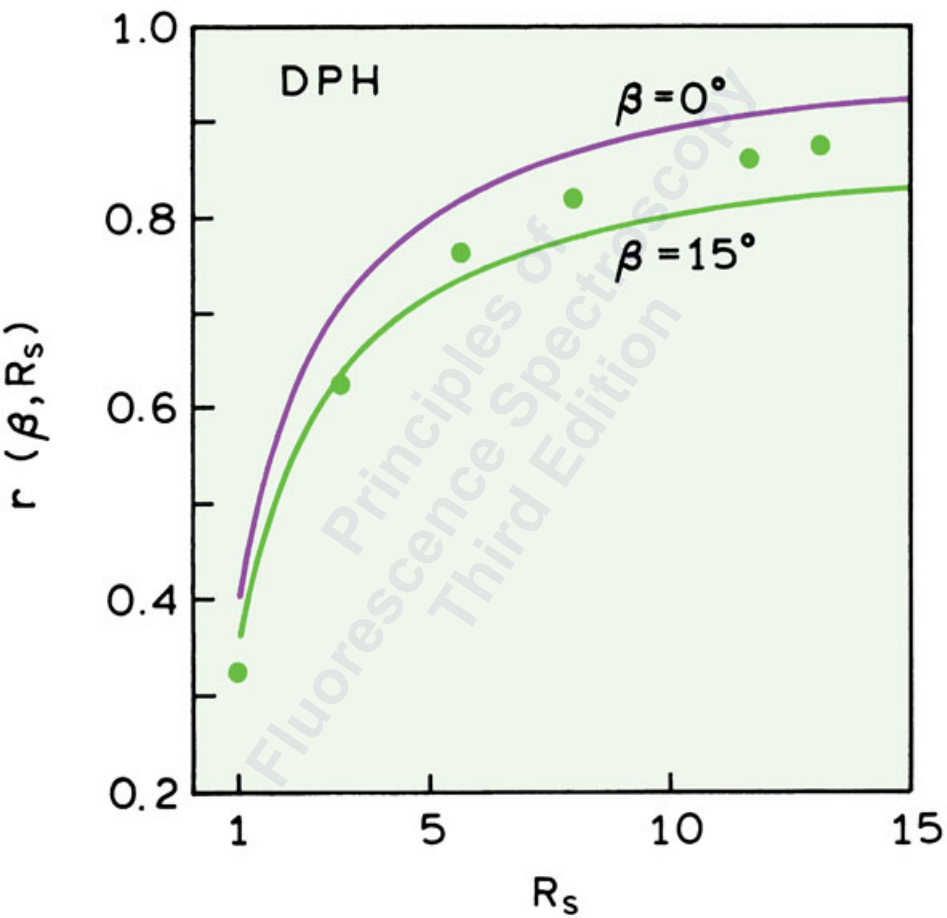


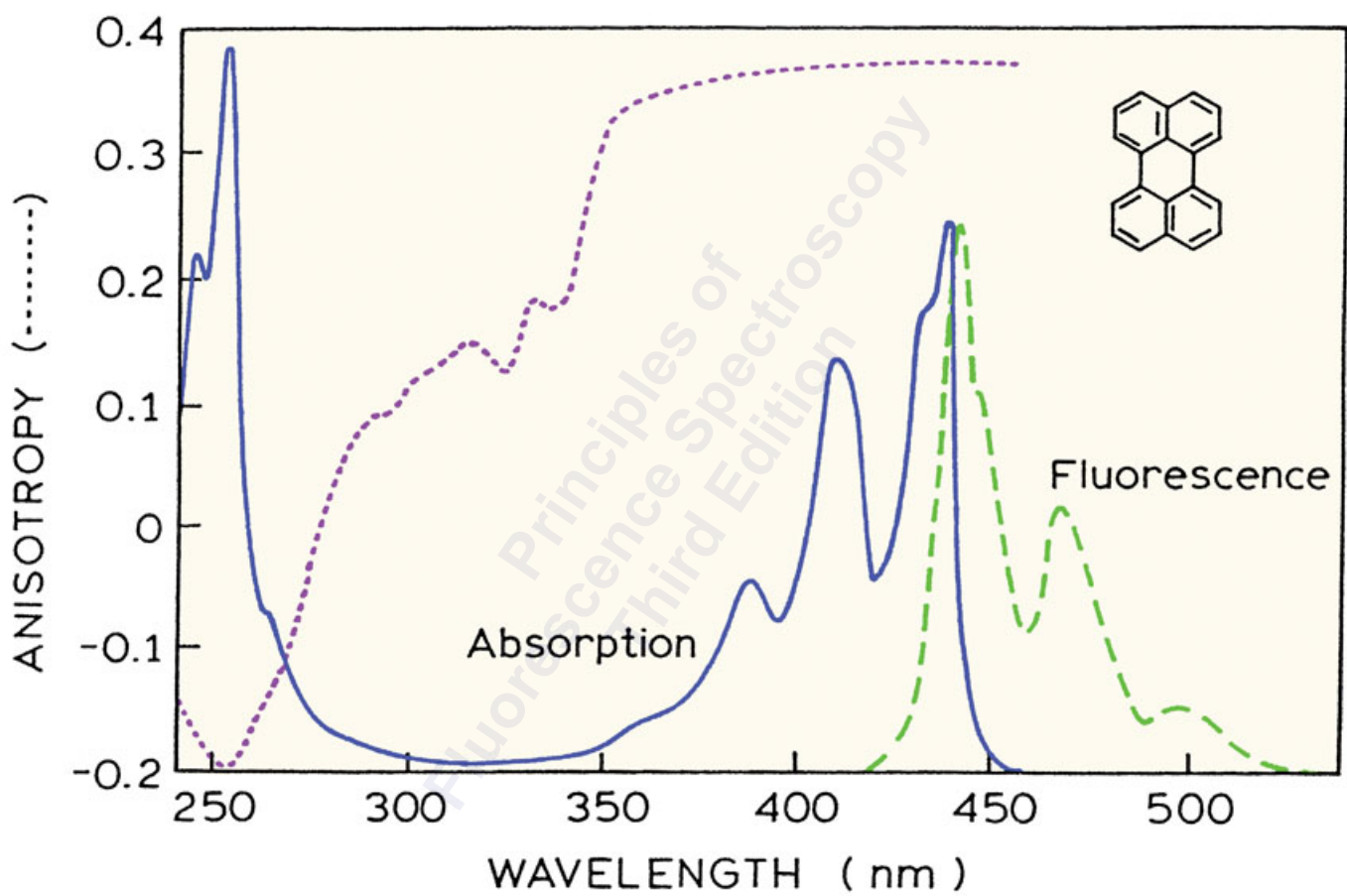


ANISOTROPY

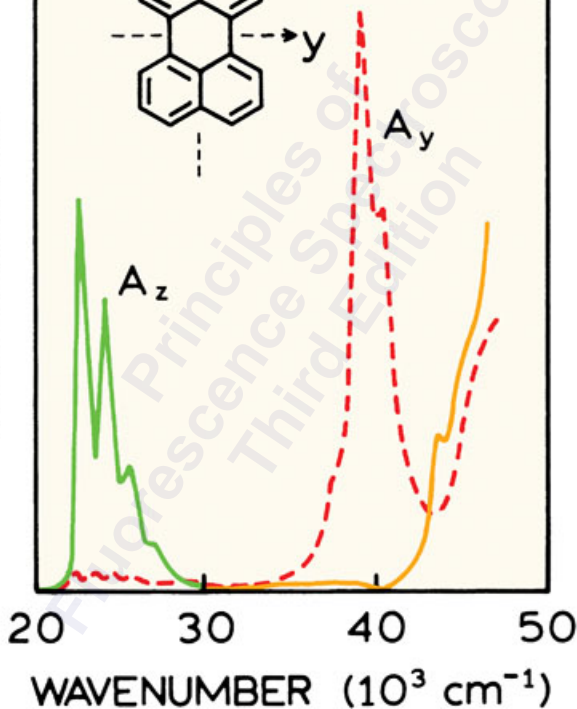
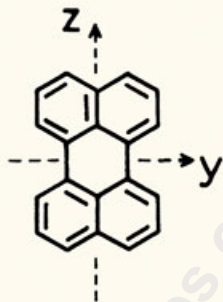




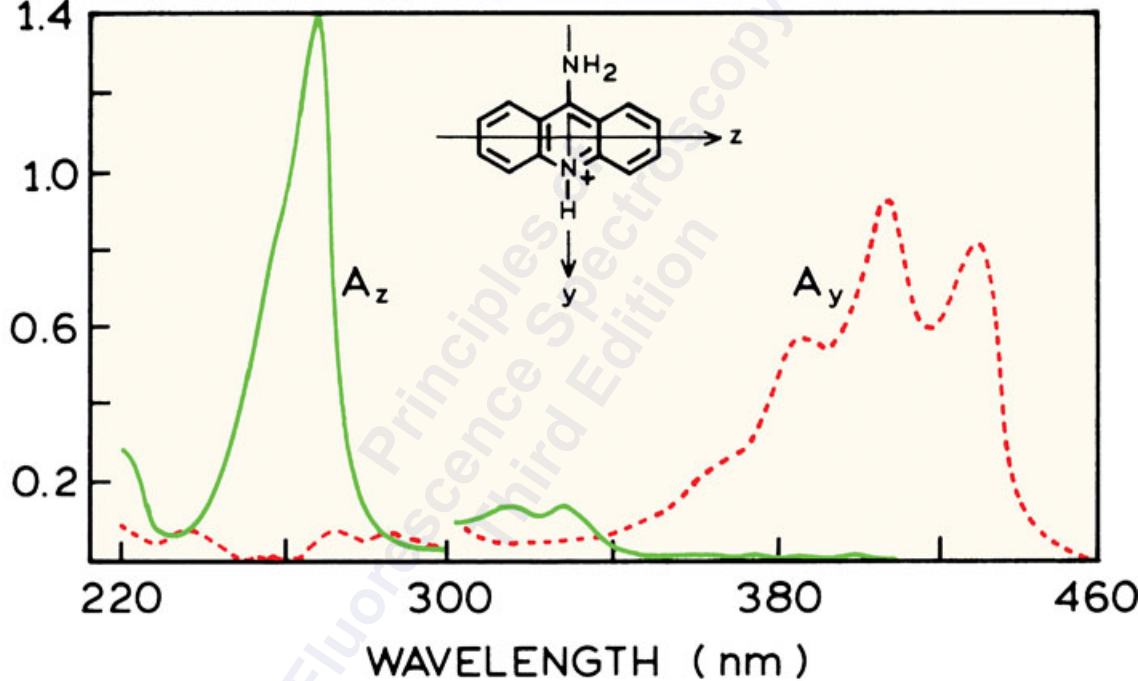




ABSORBANCE

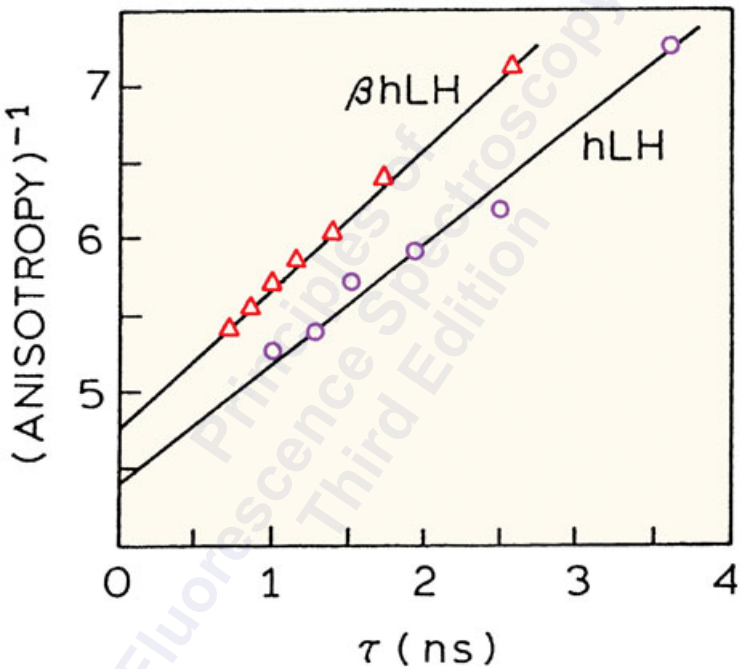


ABSORPTION

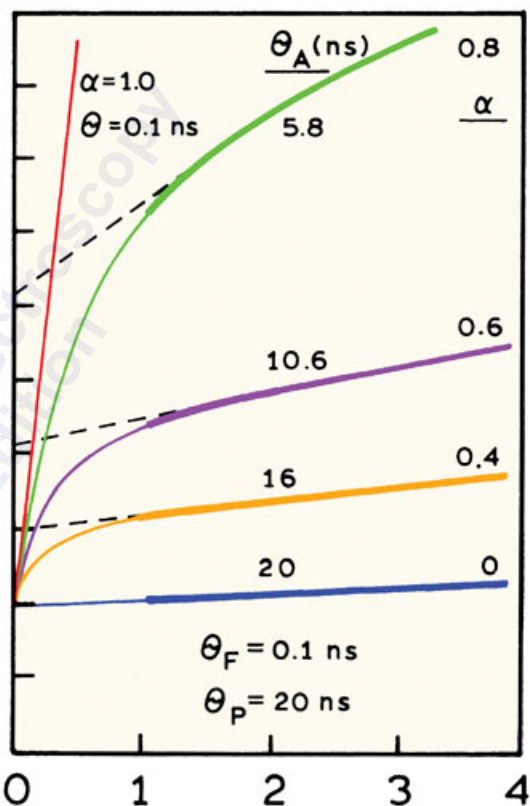
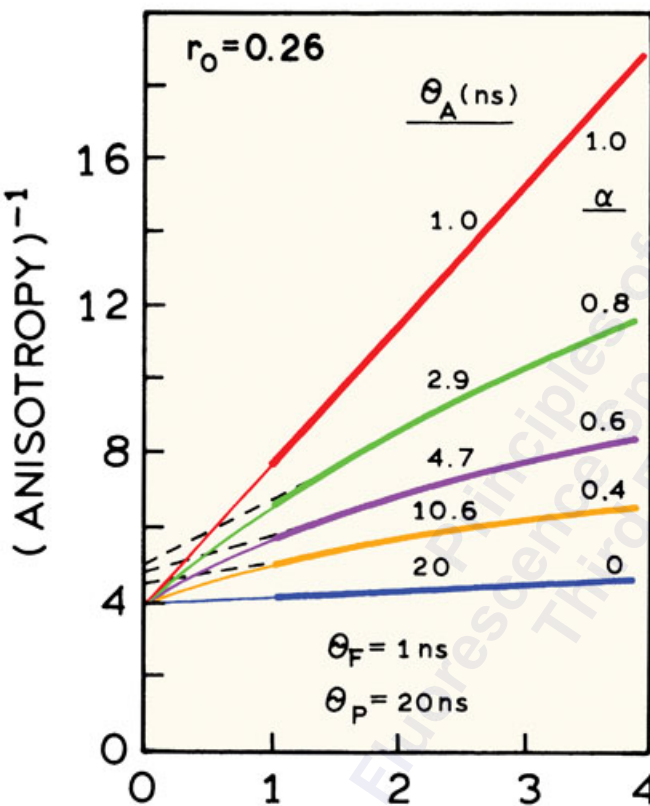


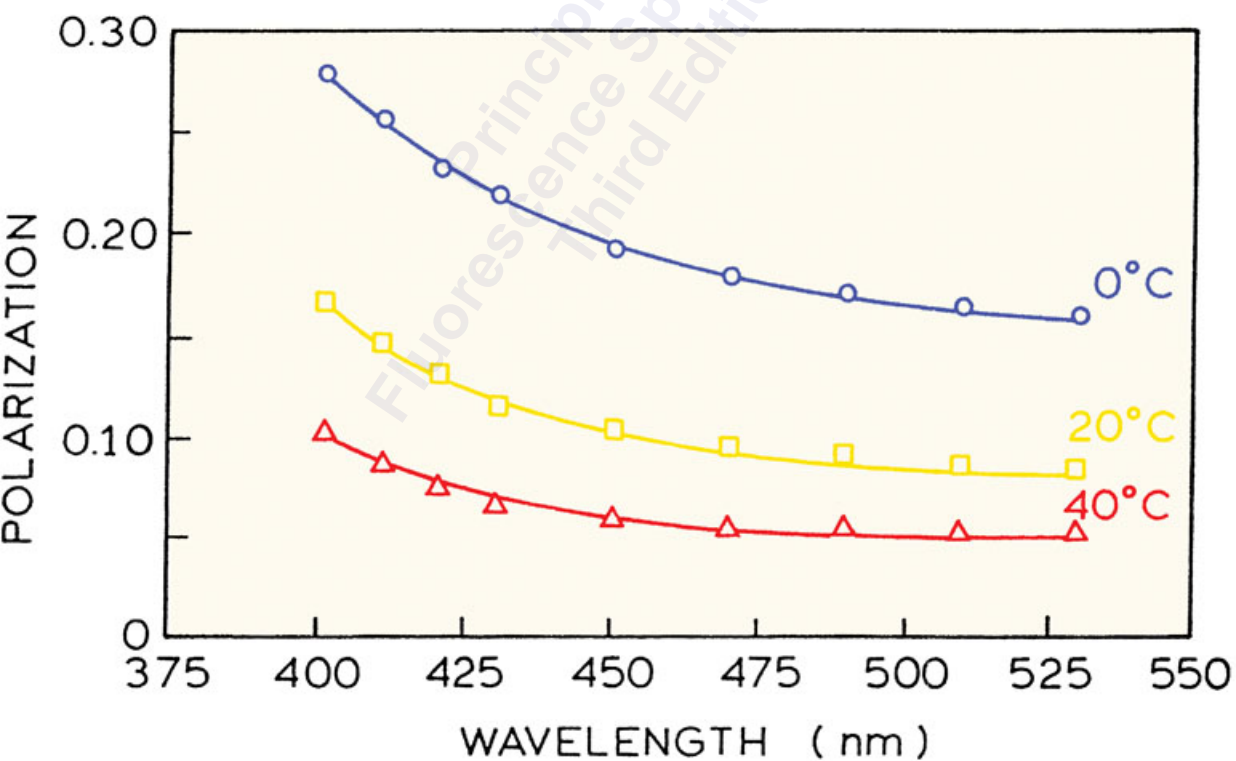
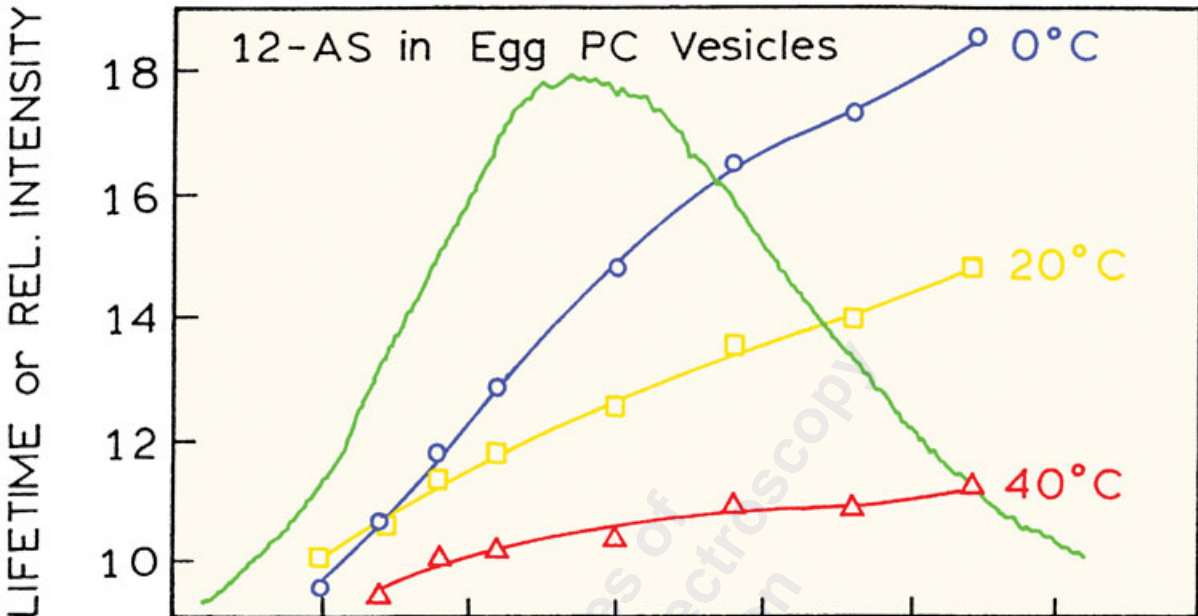


$$r_o = 0.10$$



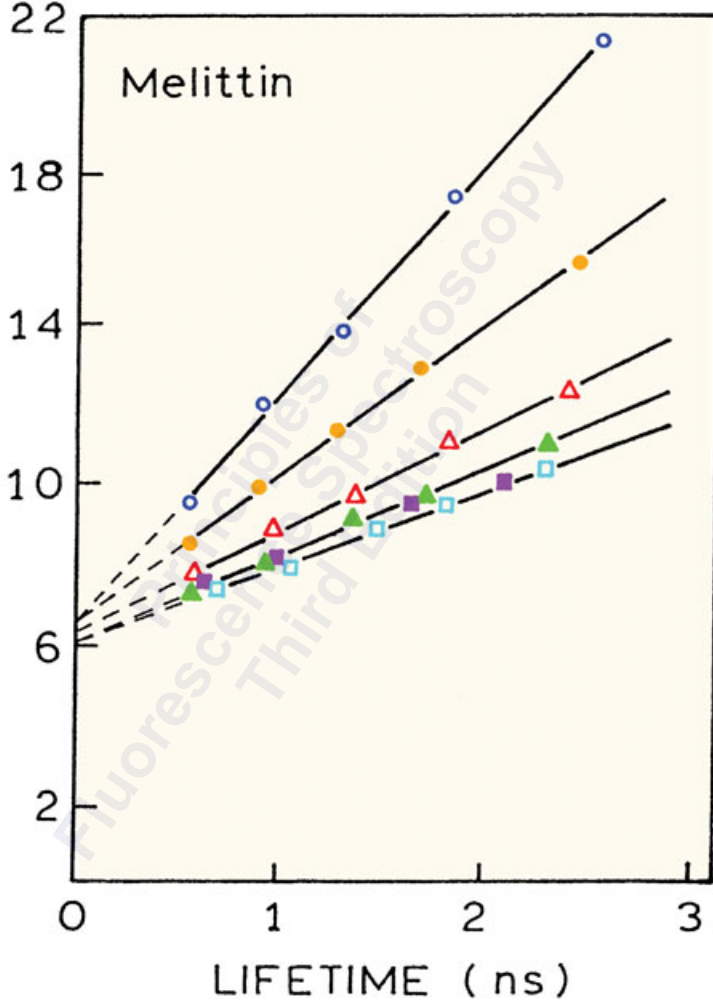




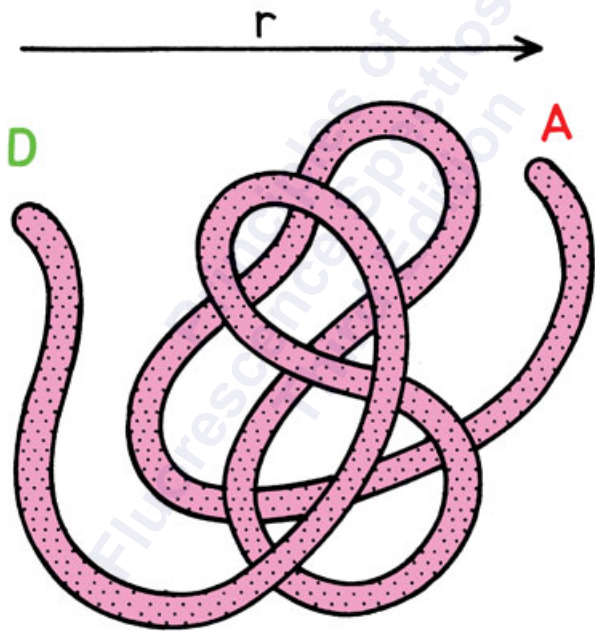


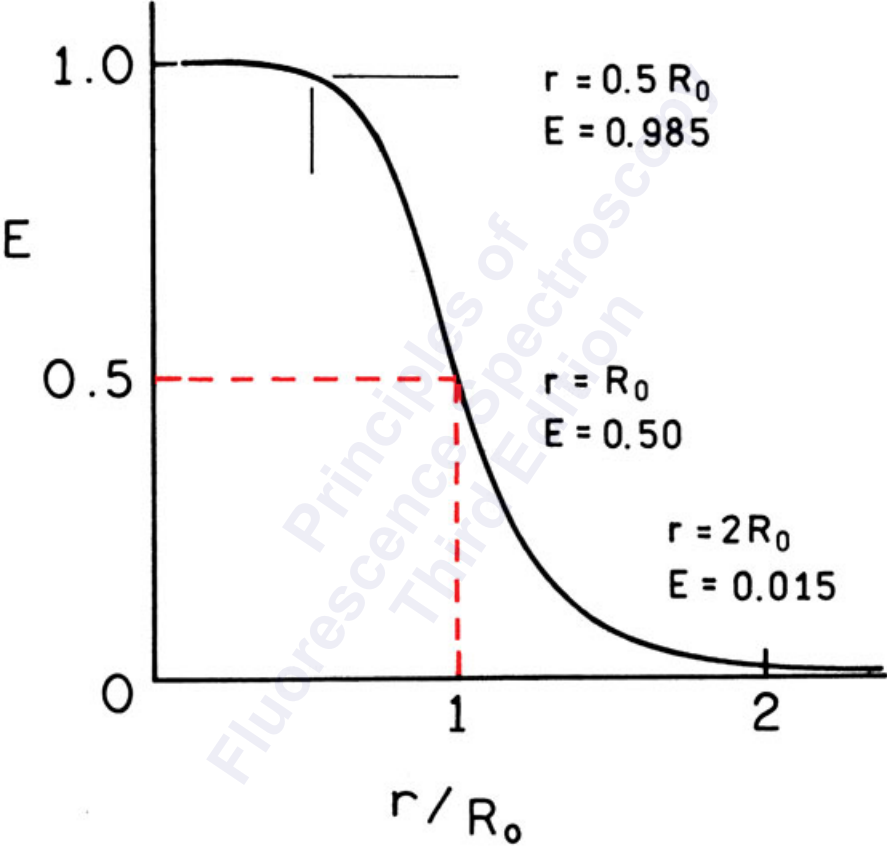
Melittin

$(\text{ANISOTROPY})^{-1}$

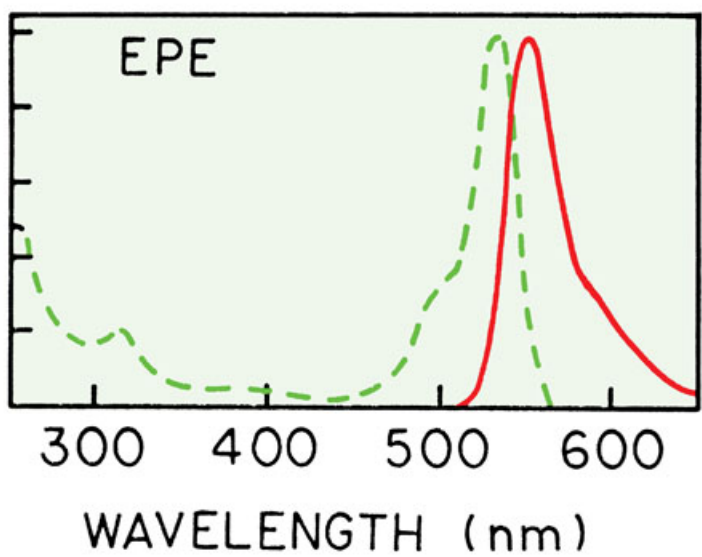
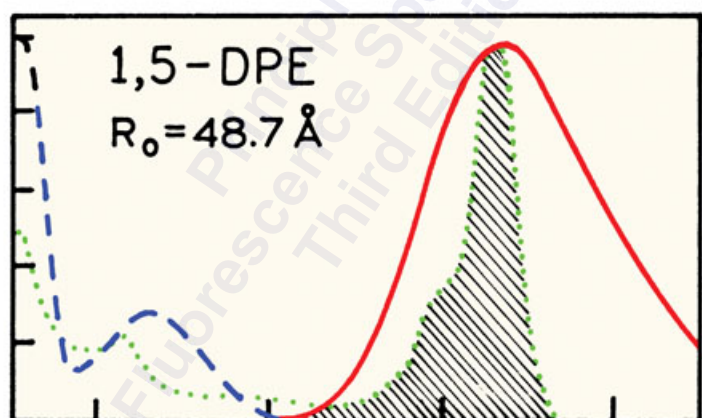
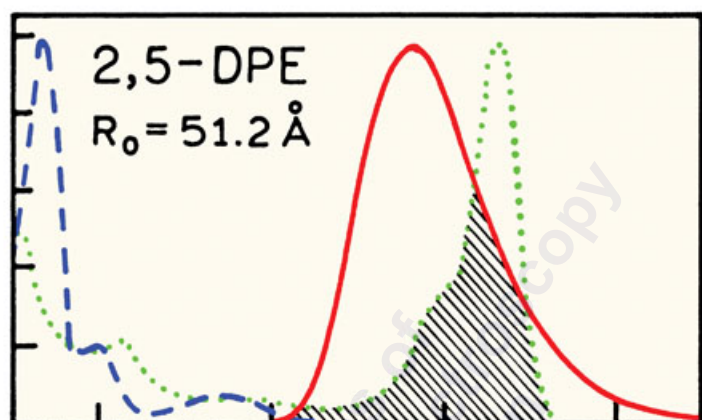
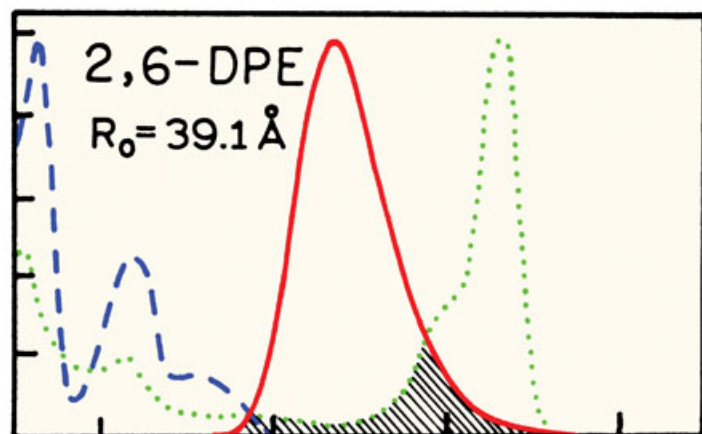


$$k_T(r) = \frac{1}{\tau_D} \left( \frac{R_0}{r} \right)^6 = \text{TRANSFER RATE}$$





EXCITATION (---) or EMISSION (—)

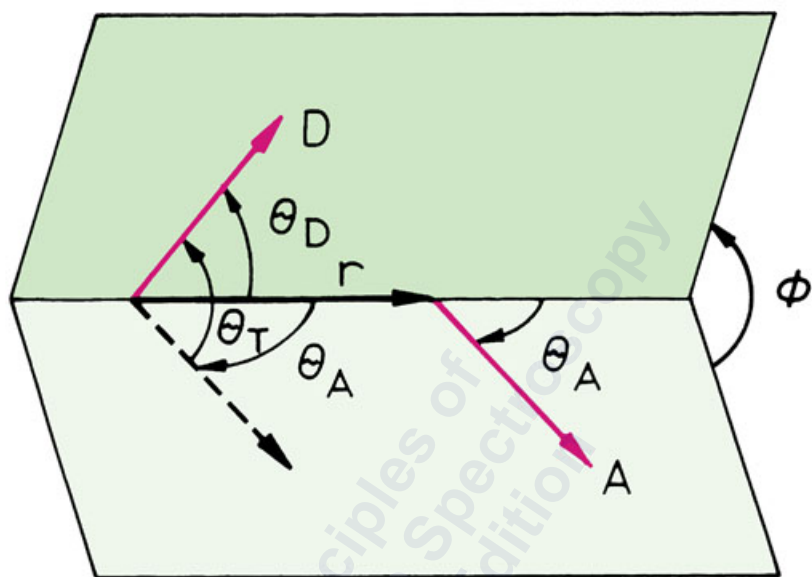


# THEODOR FÖRSTER

15. 5. 1910 – 20. 5. 1974



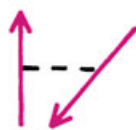




$$\chi^2 = 4$$



$$\chi^2 = 1$$

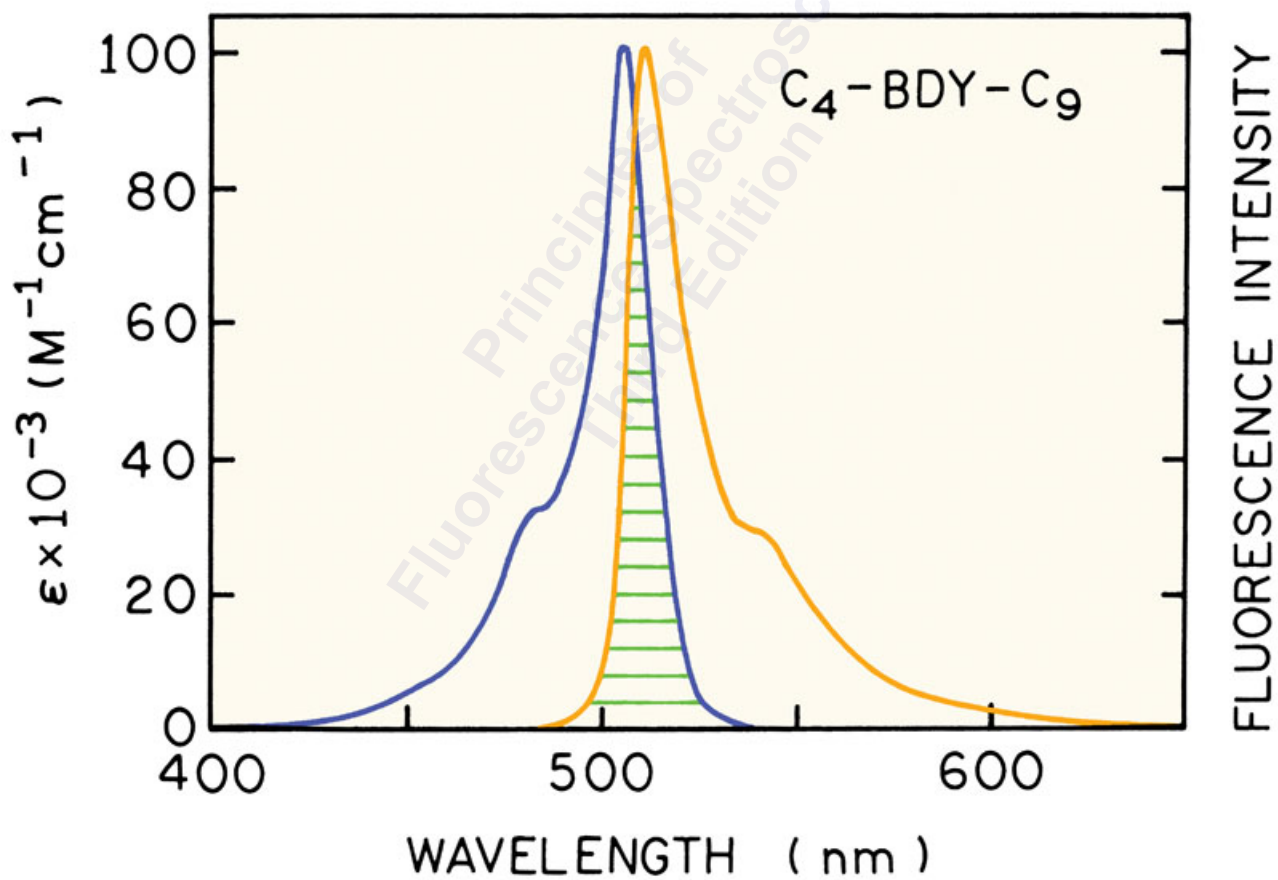
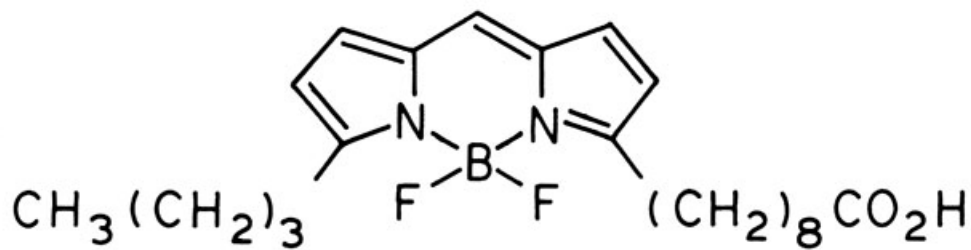


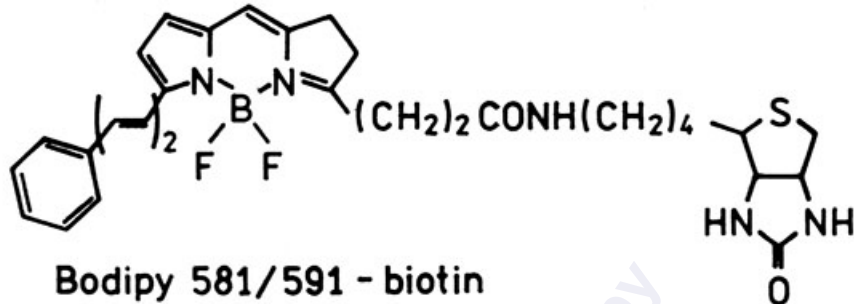
$$\chi^2 = 0$$

$$\chi^2 = (\cos \theta_T - 3 \cos \theta_D \cos \theta_A)^2$$

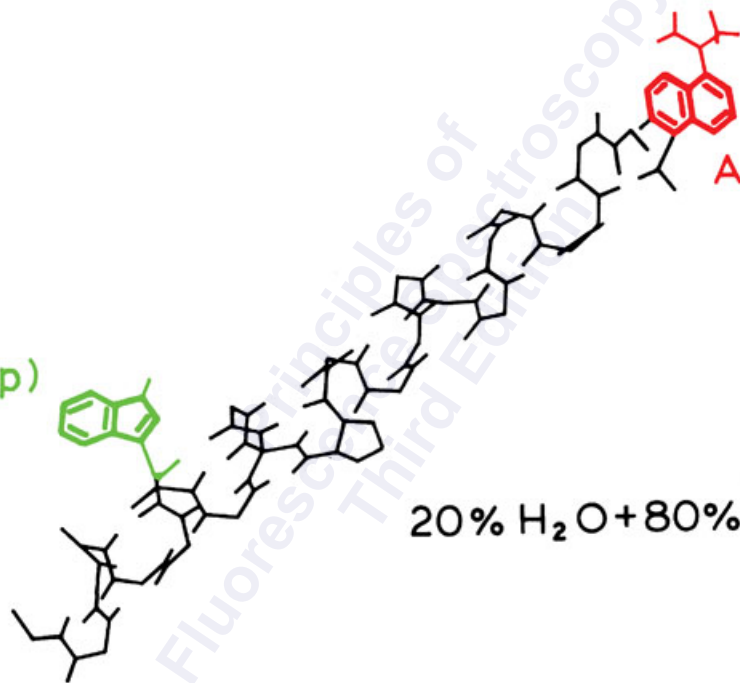
$$\chi^2 = (\sin \theta_D \sin \theta_A \cos \phi - 2 \cos \theta_D \cos \theta_A)^2$$





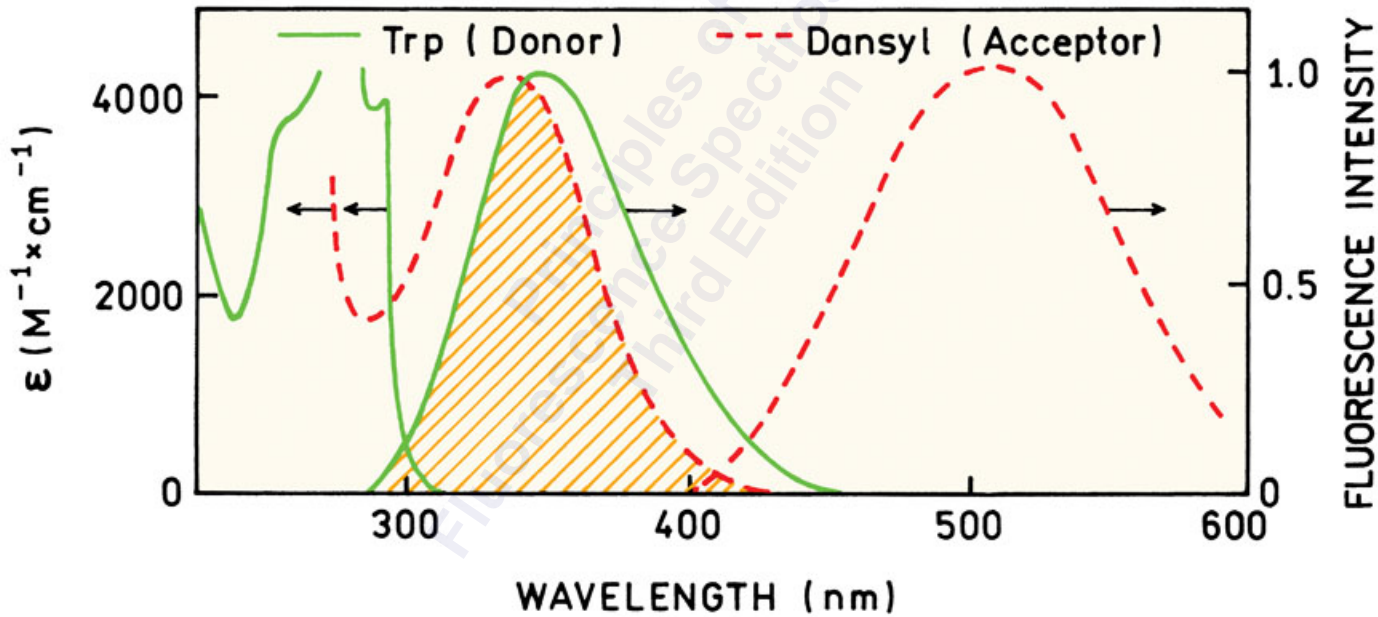


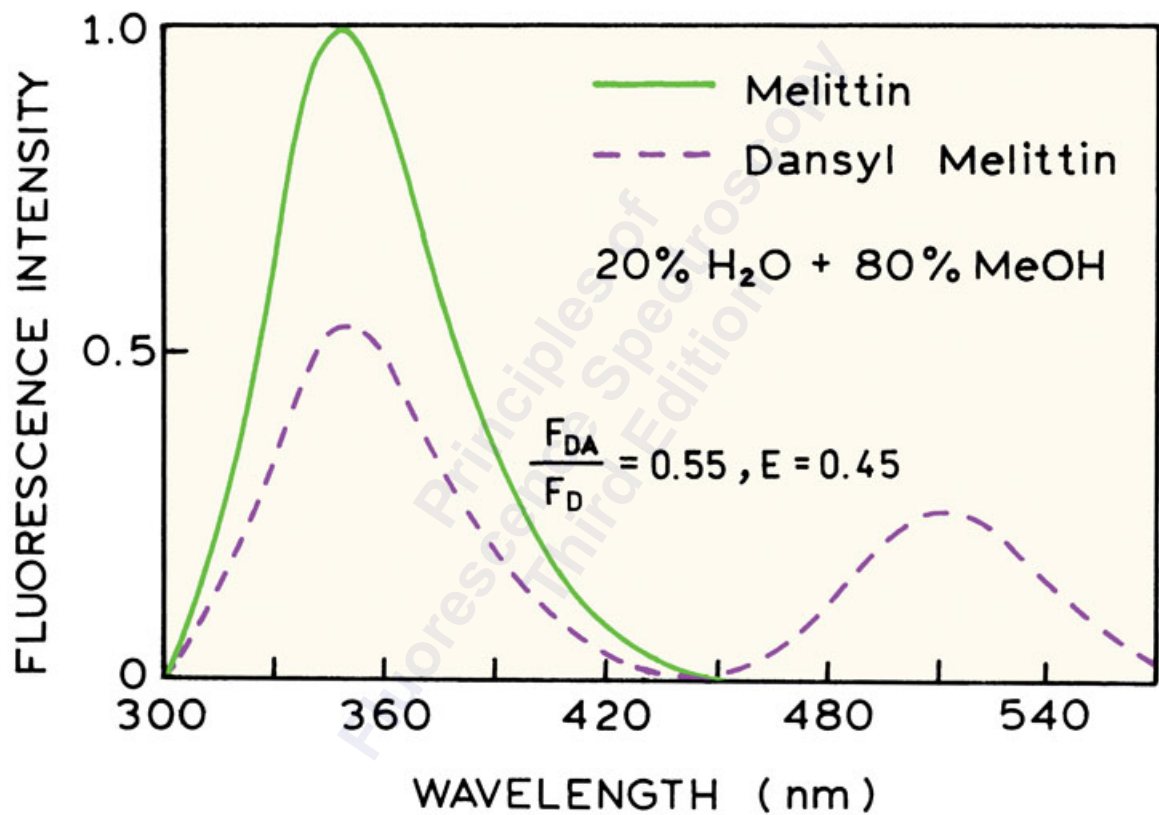
D (Trp)



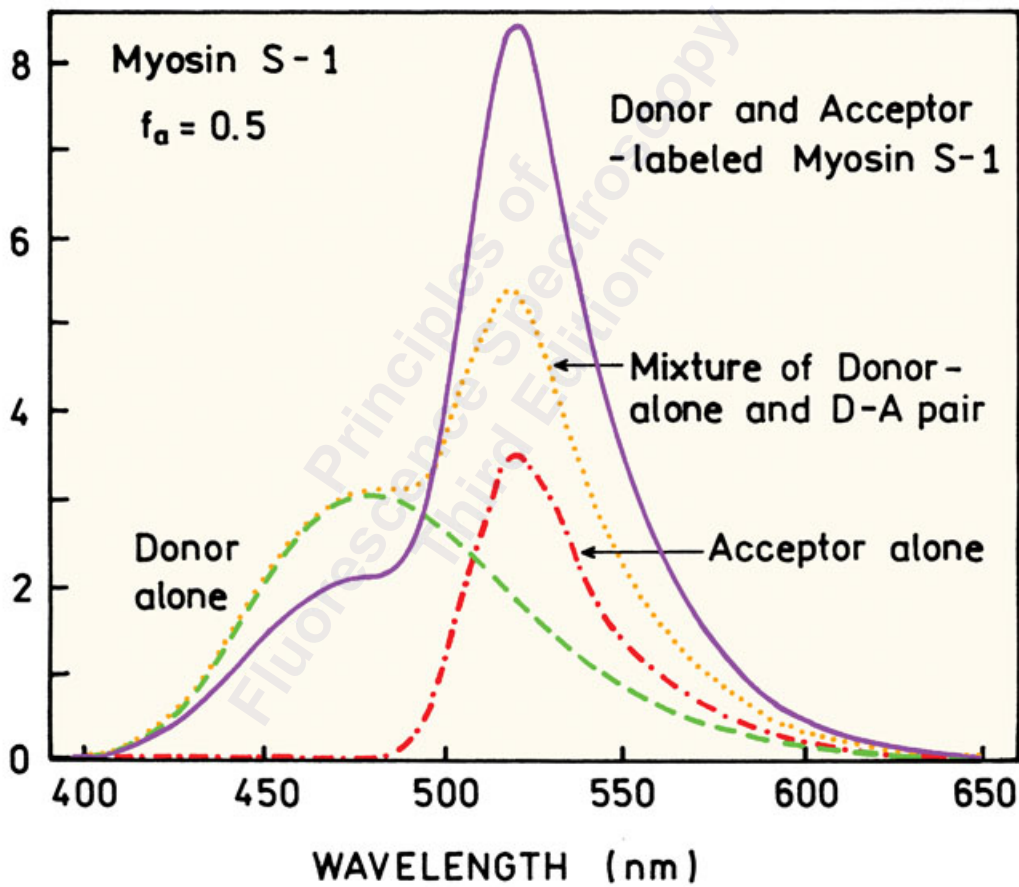
A (Dansyl)

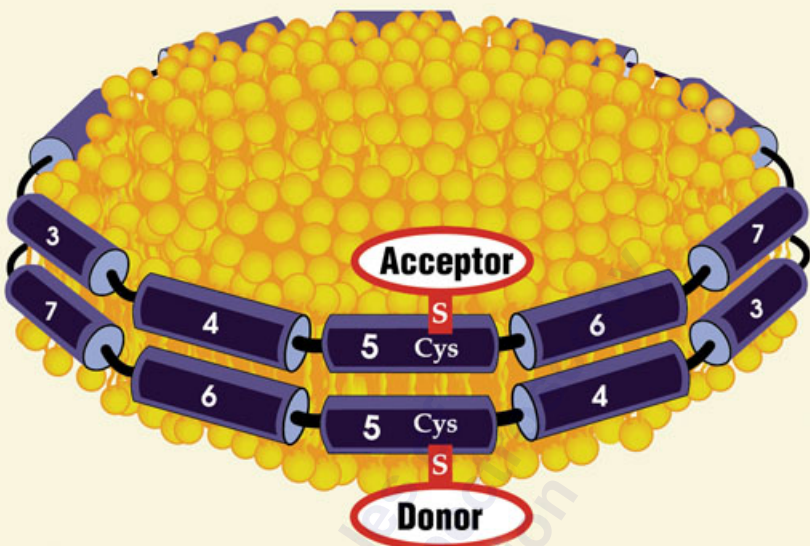
20% H<sub>2</sub>O + 80% MeOH





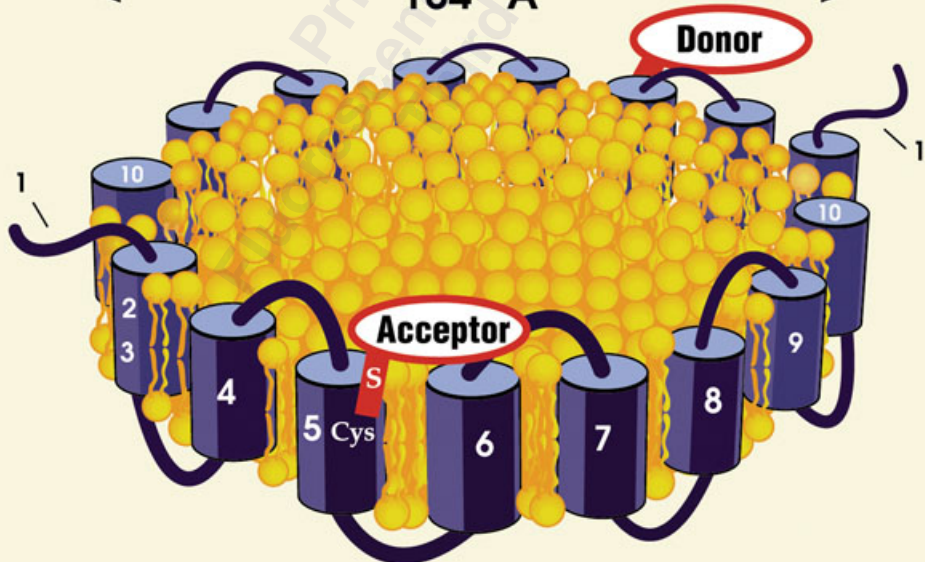
FLUORESCENCE INTENSITY





"Belt" Conformation

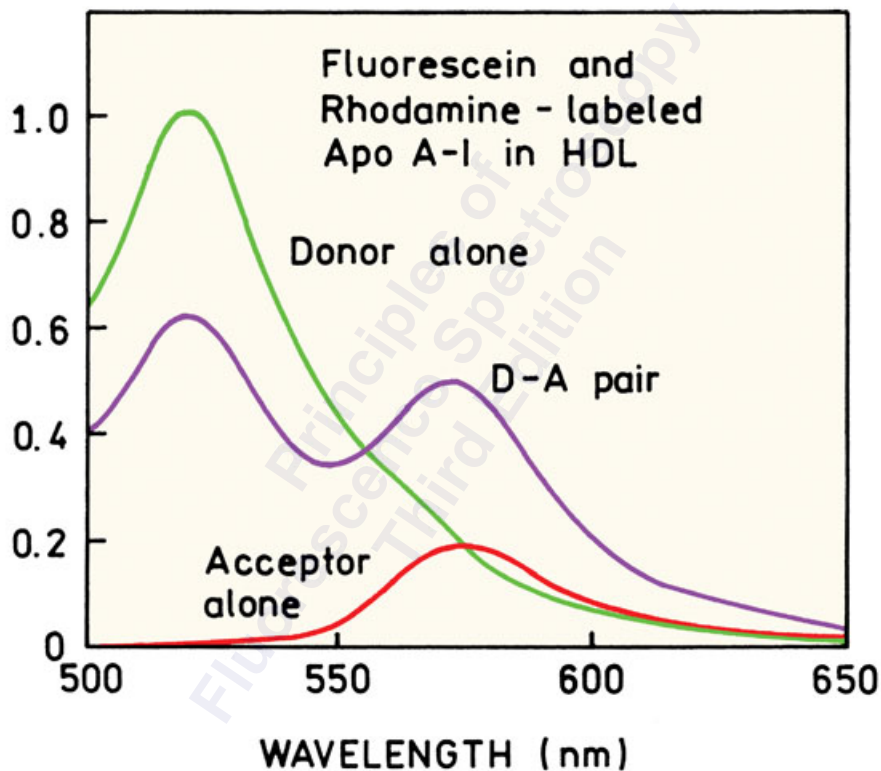
104 Å



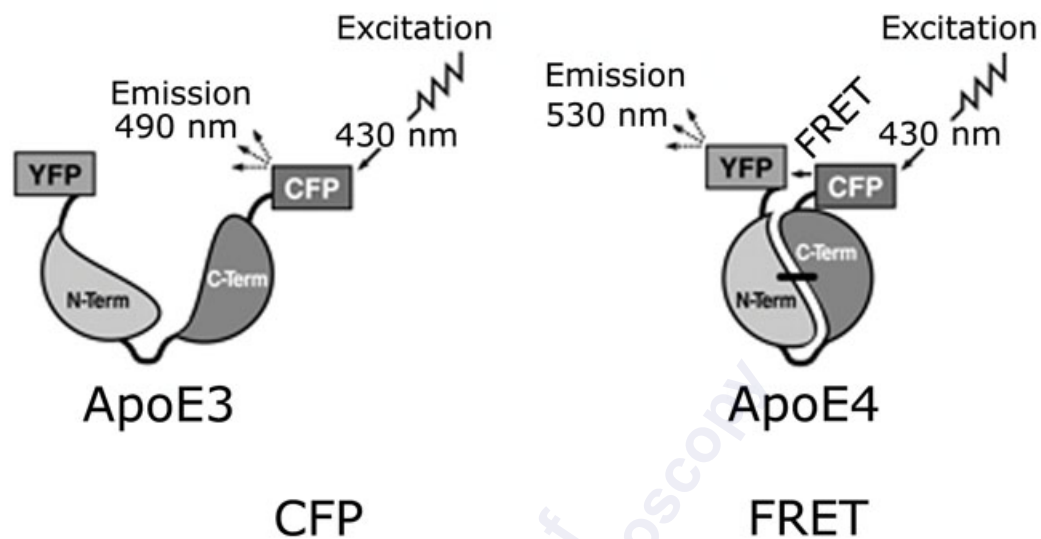
"Picket-Fence" Conformation



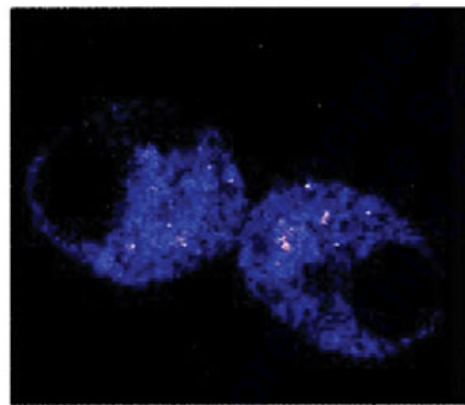
FLUORESCENCE INTENSITY



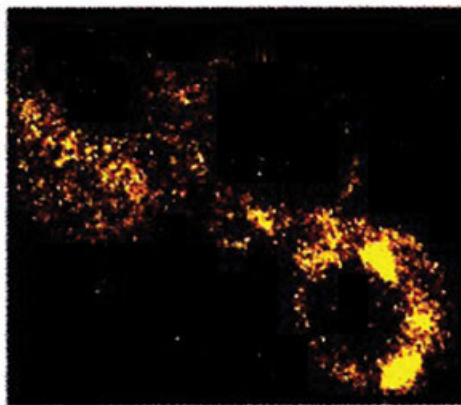
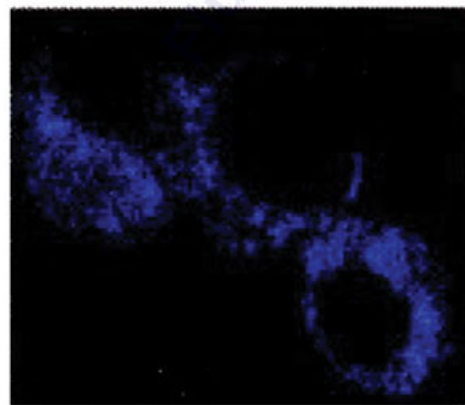


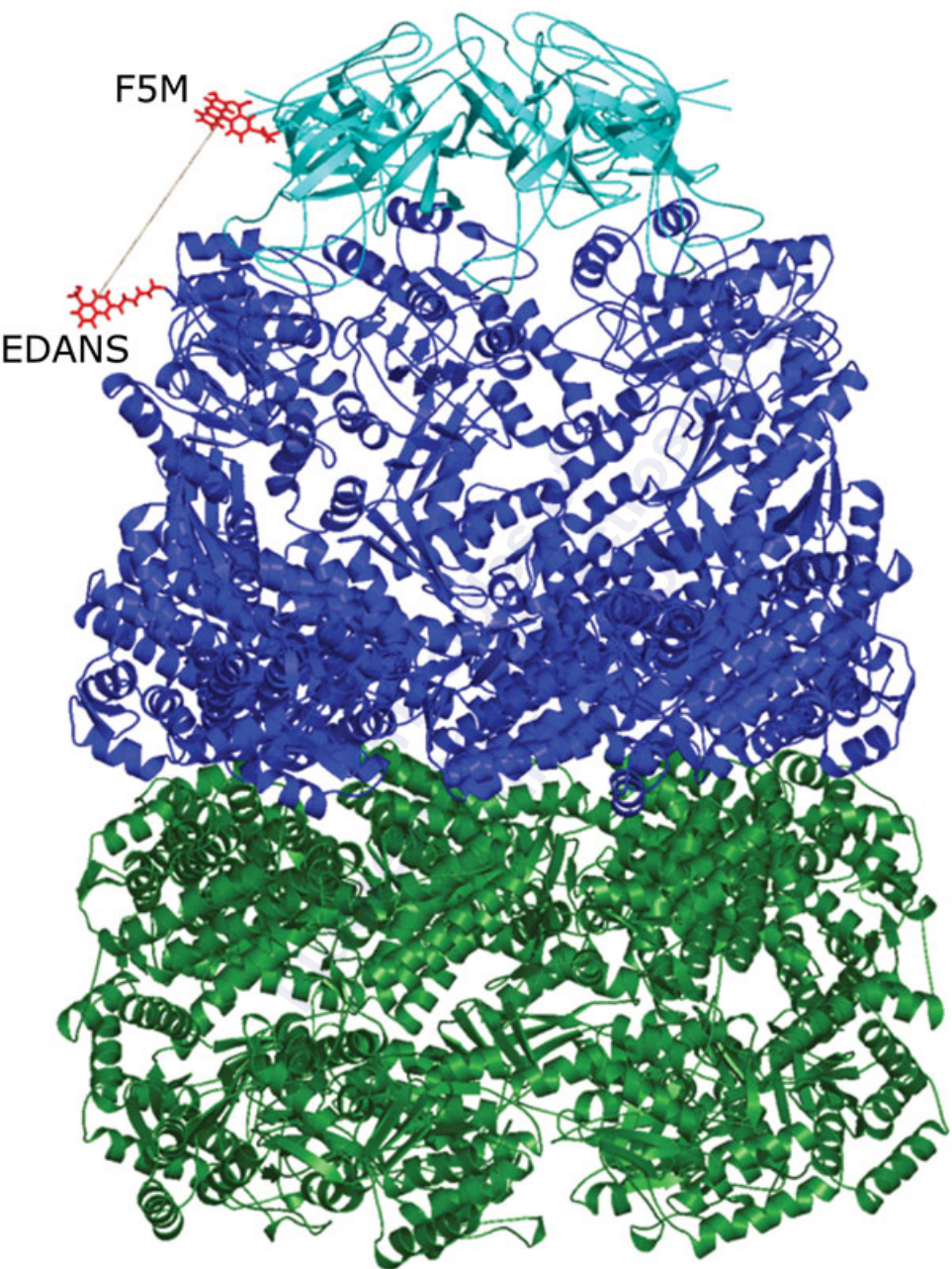


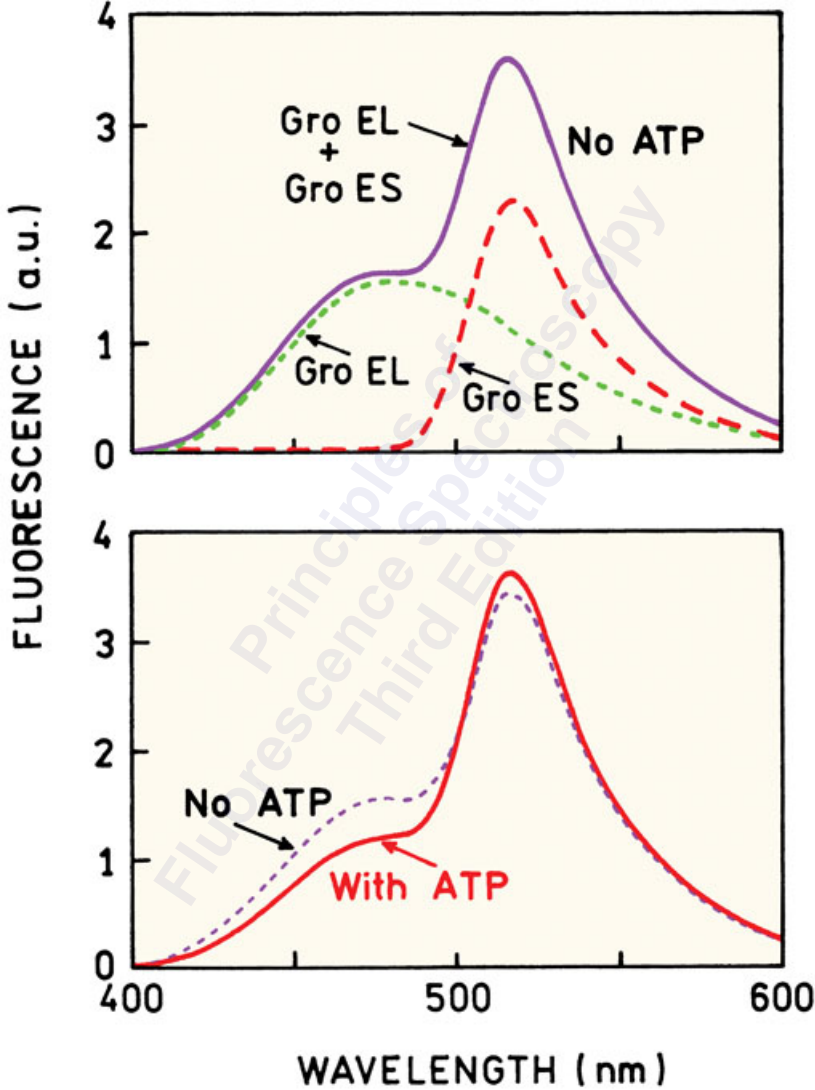
YFP-ApoE3-CFP

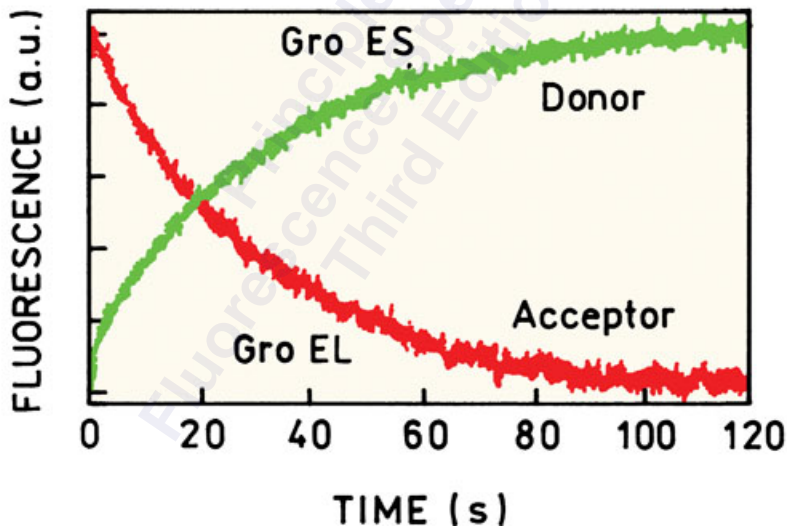
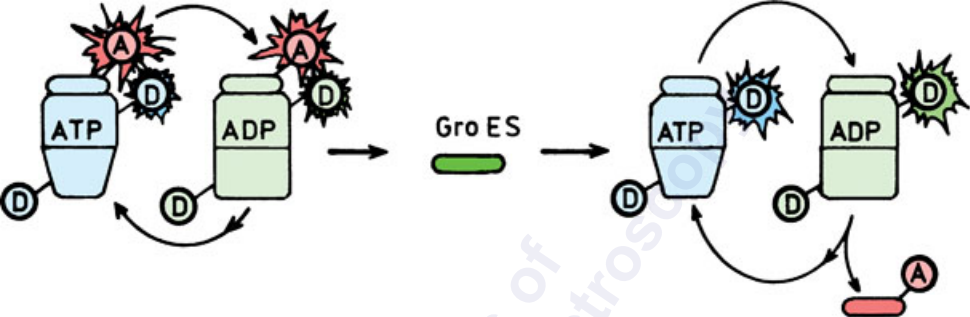


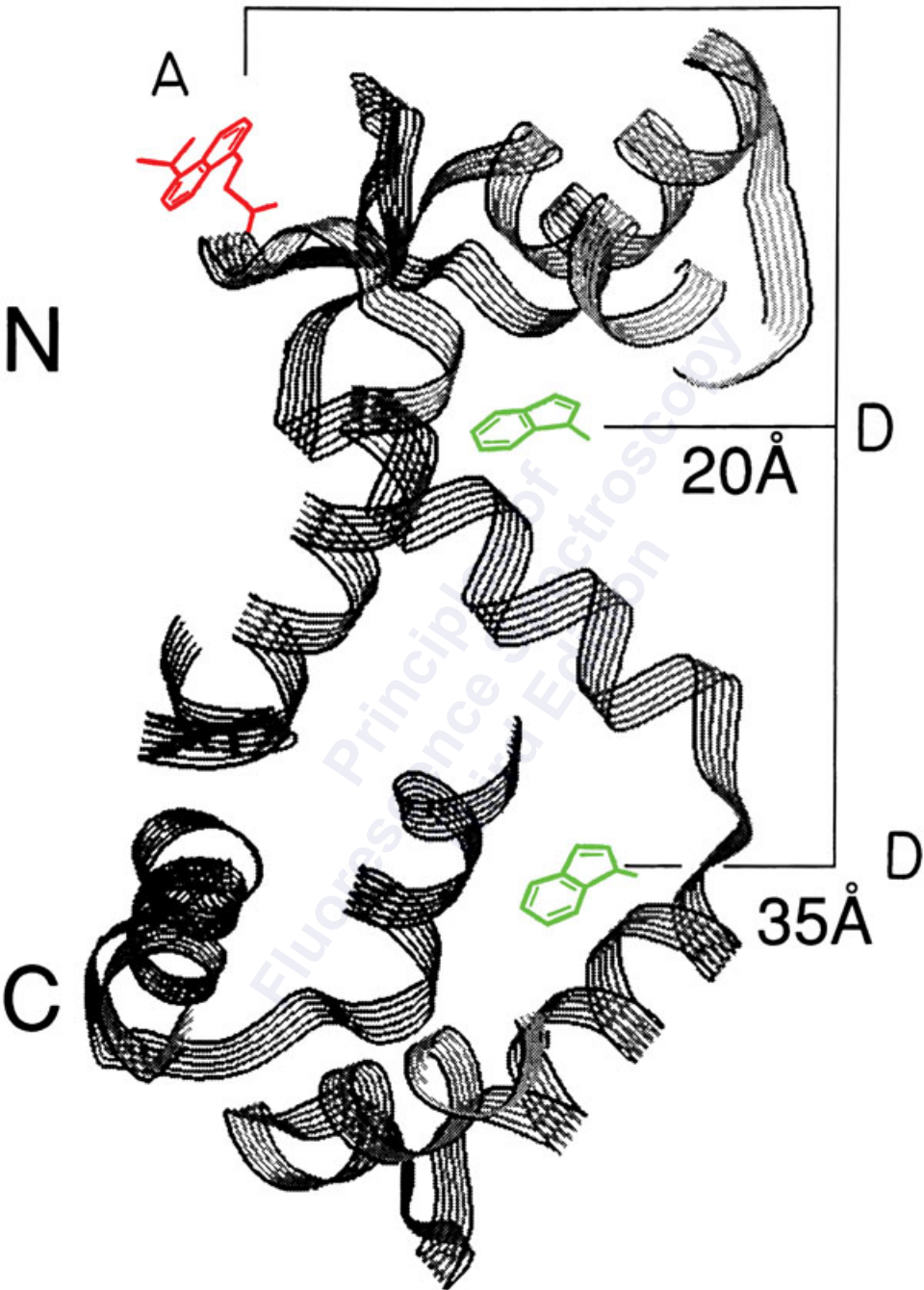
YFP-ApoE4-CFP





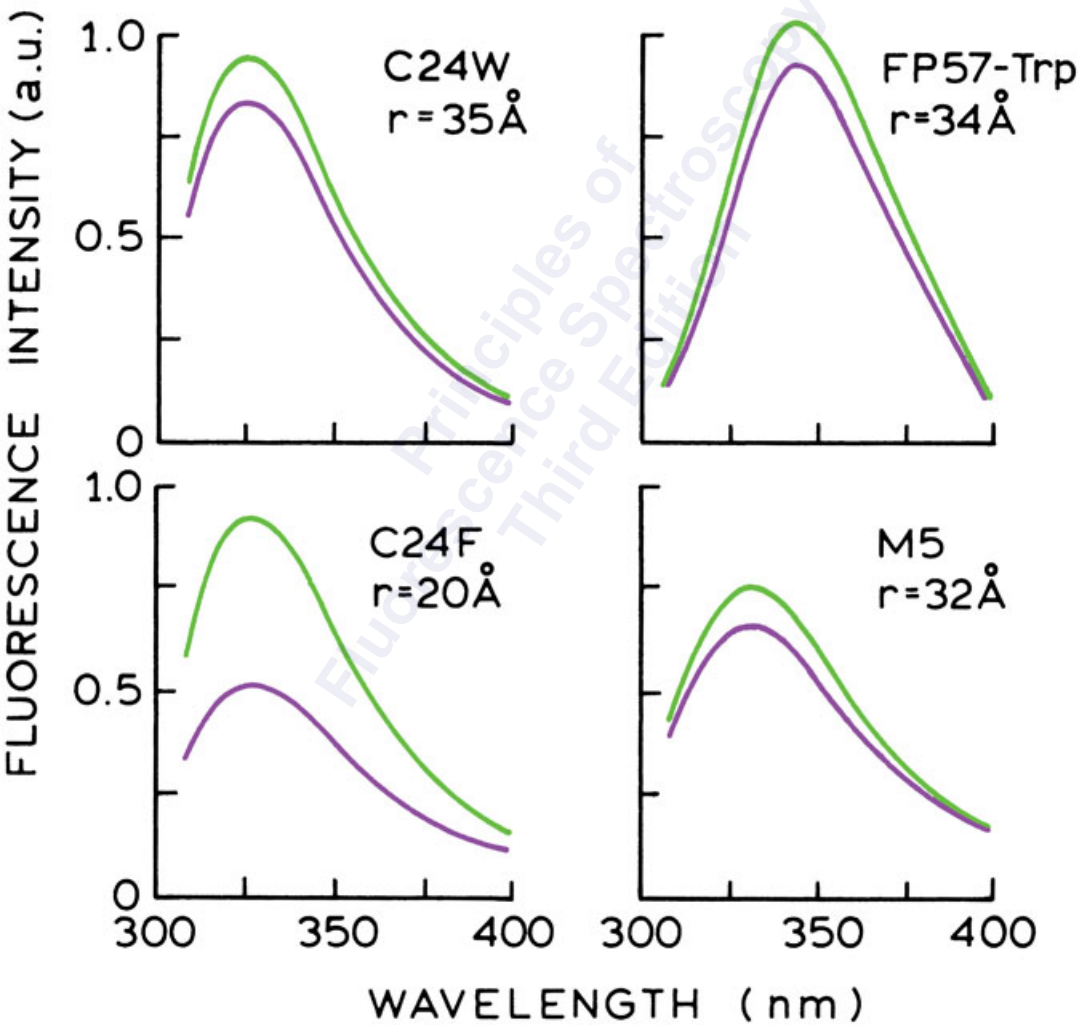


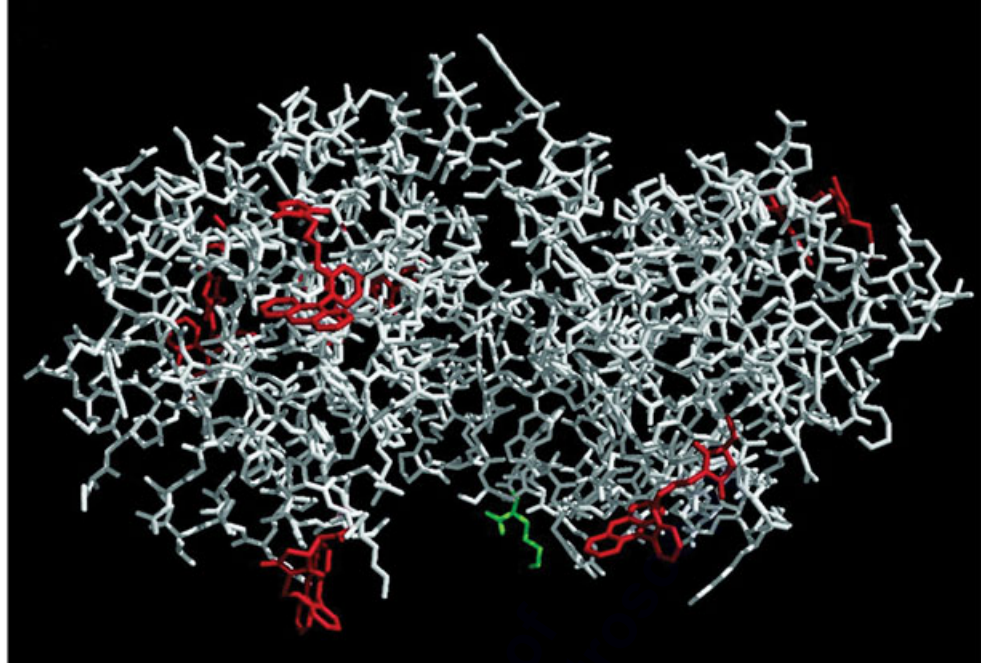




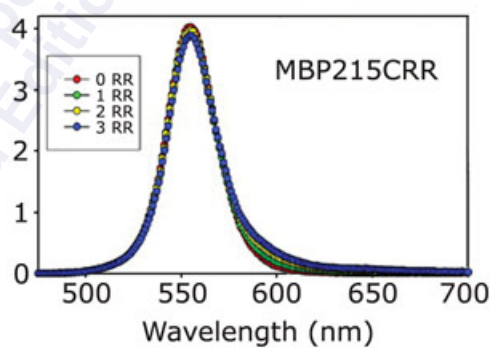
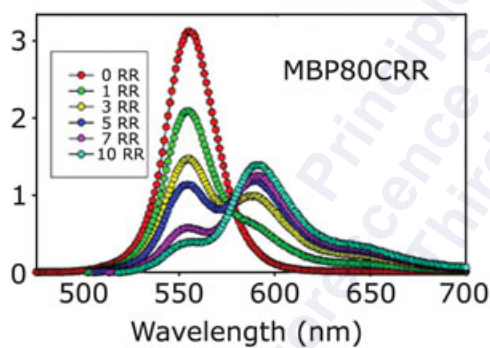


M 5	KRR <u>W</u> KKNFIAVSAANRFG
C24W	QIL <u>W</u> FRGLNRIQTQIRVVNAFRSS
C24F	QILFFRGLNRIQTQIRVVNA <u>W</u> RSS
FP57-Trp	QAS <u>W</u> RGHITRKKLKGEK

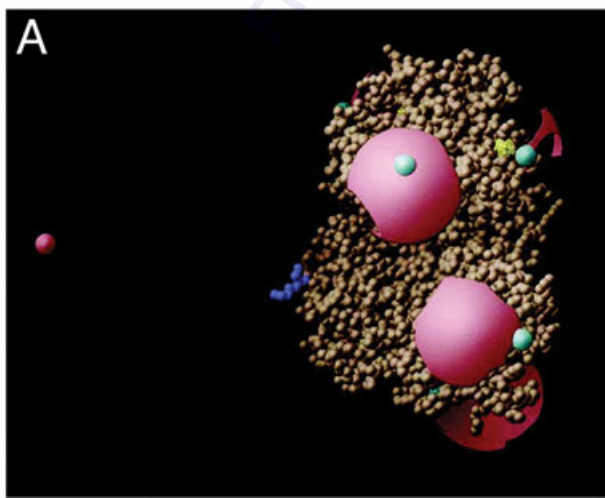




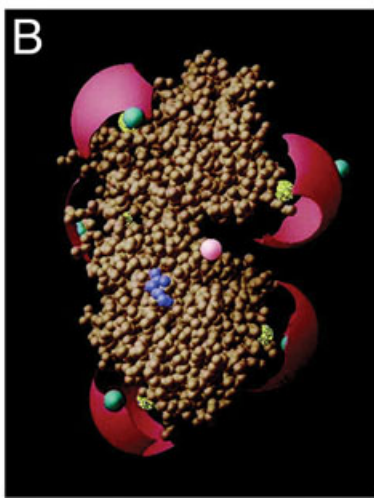
Photoluminescence (a.u.)

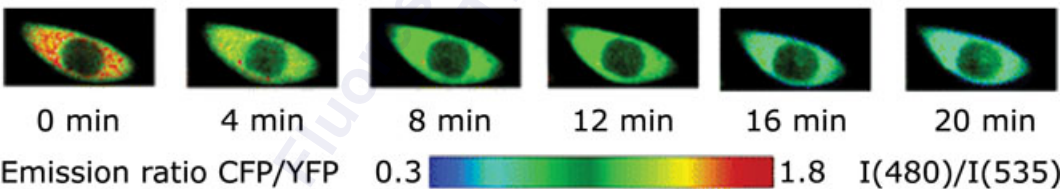
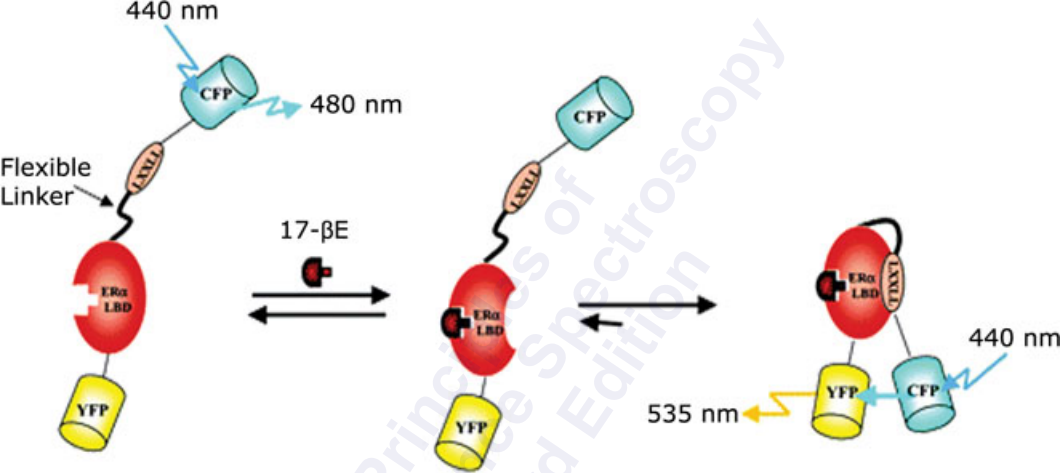


**A**

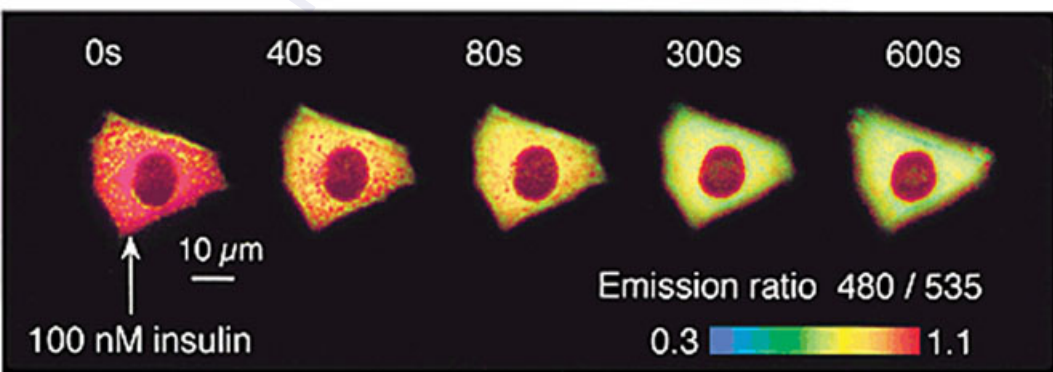
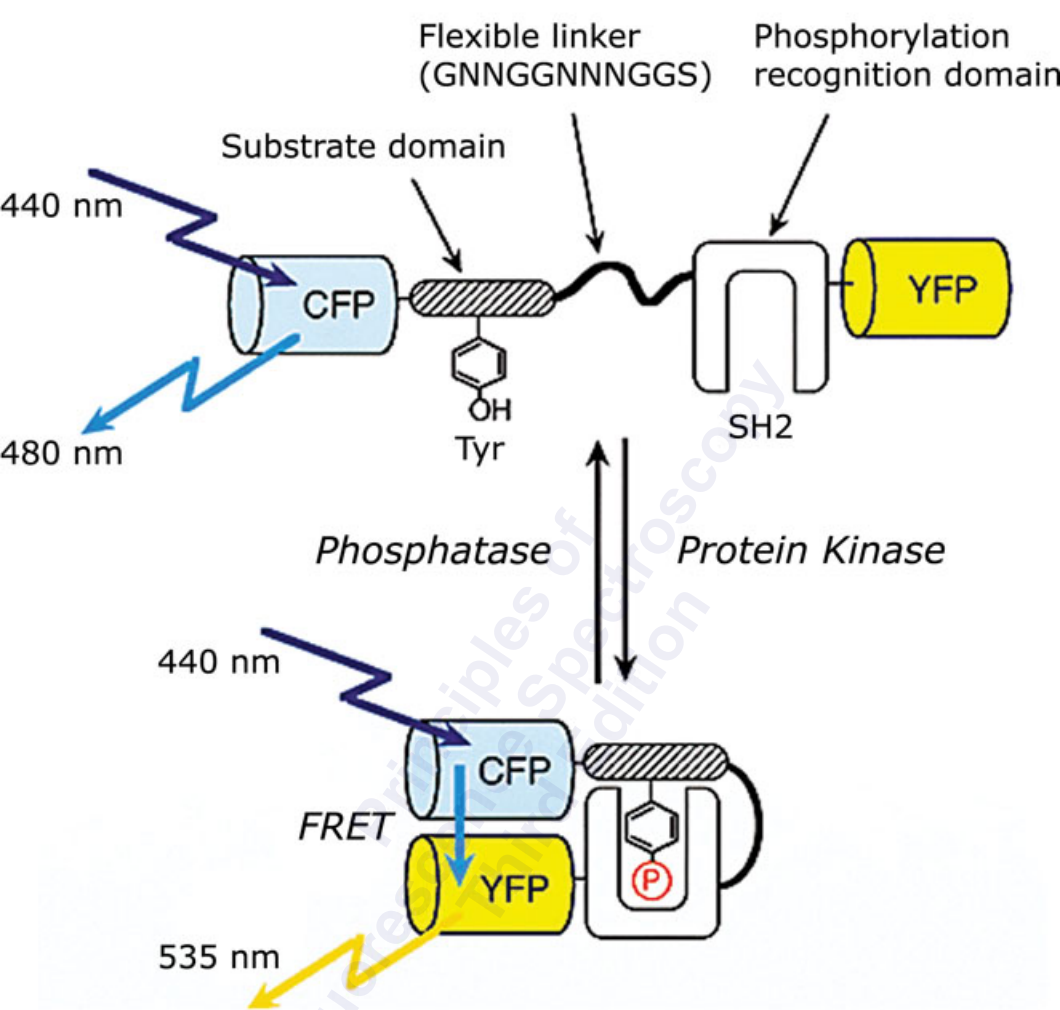


**B**

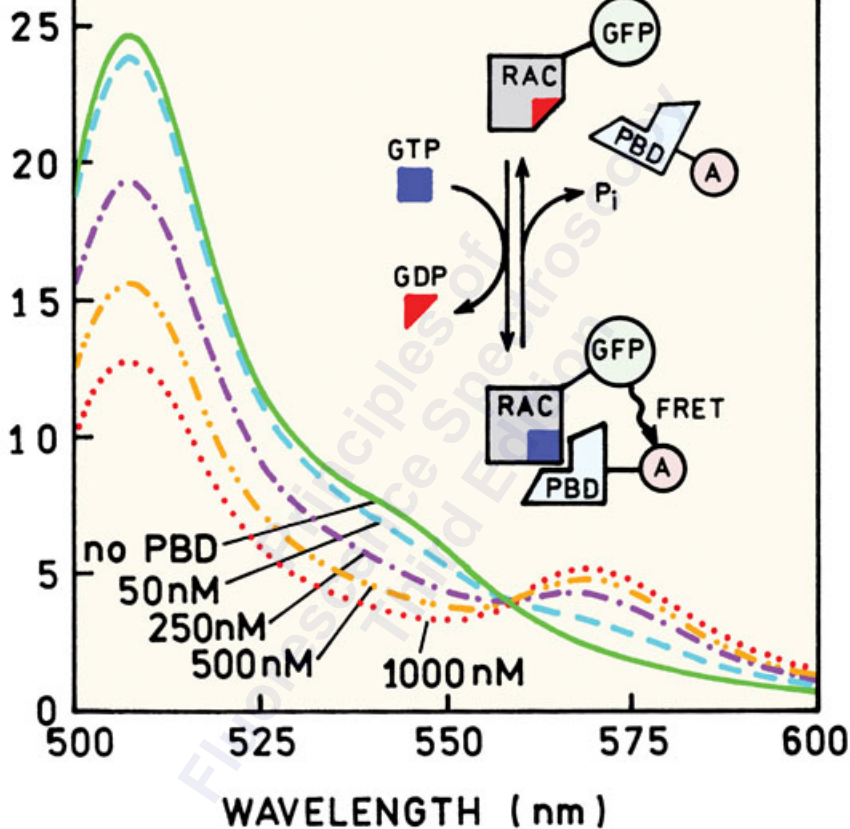








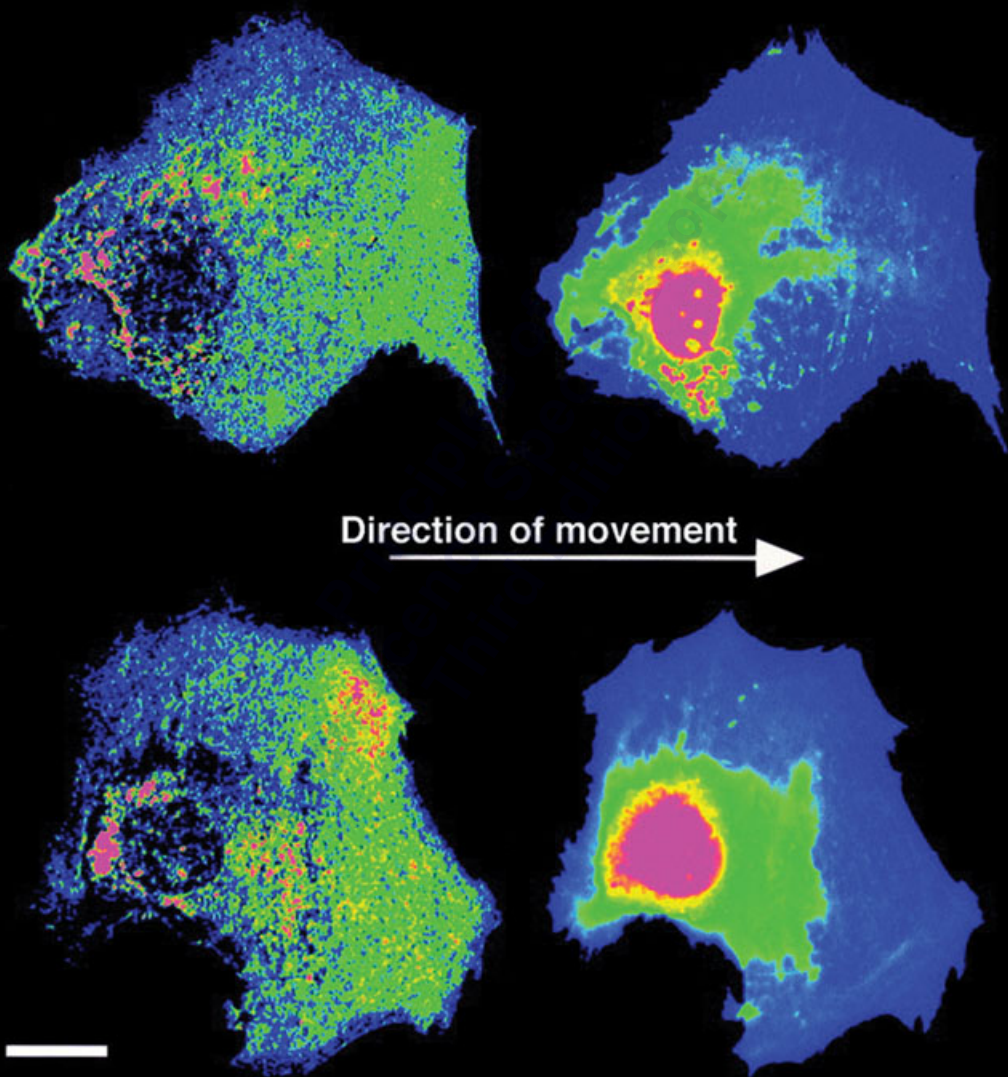
EMISSION INTENSITY



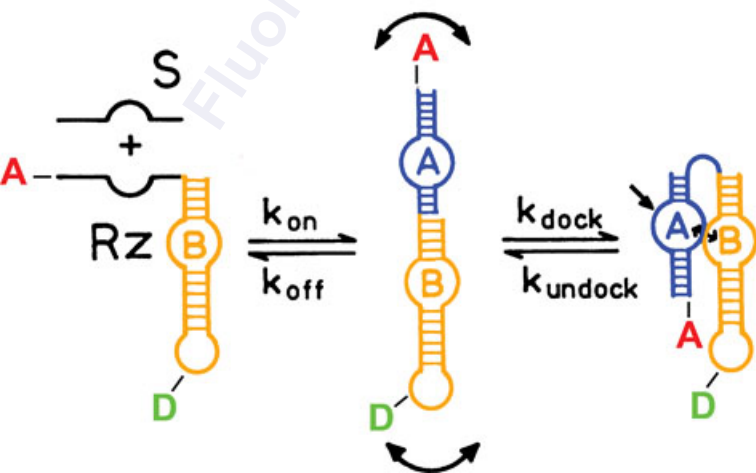
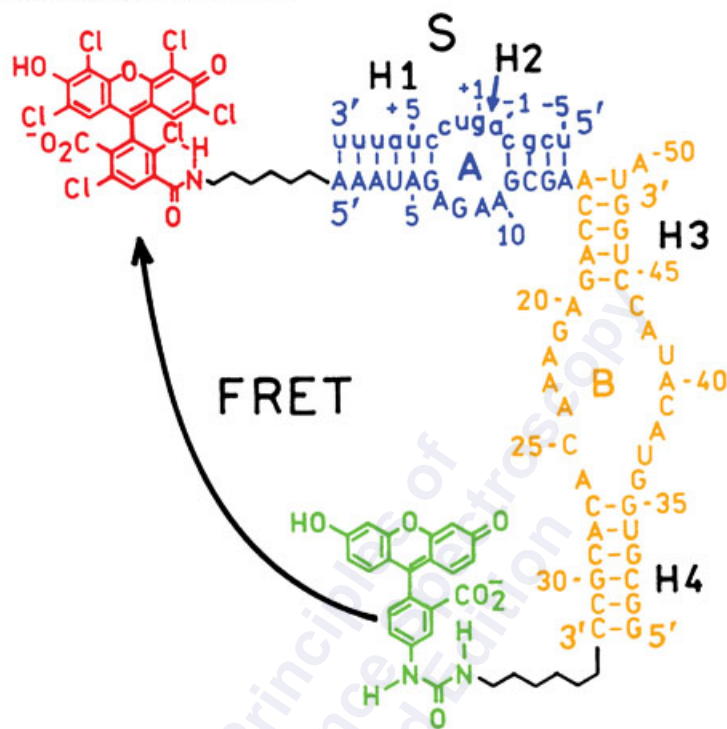
FRET

GFP-Rac

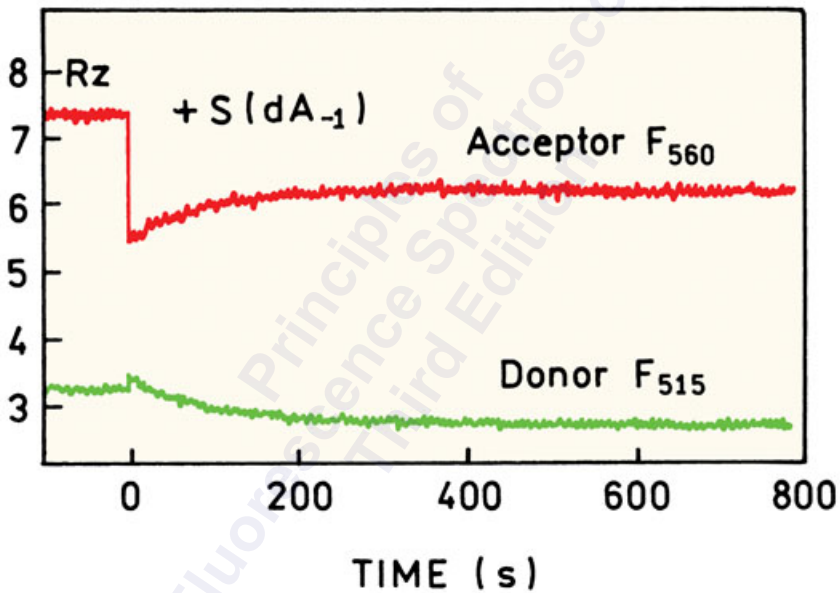
Direction of movement

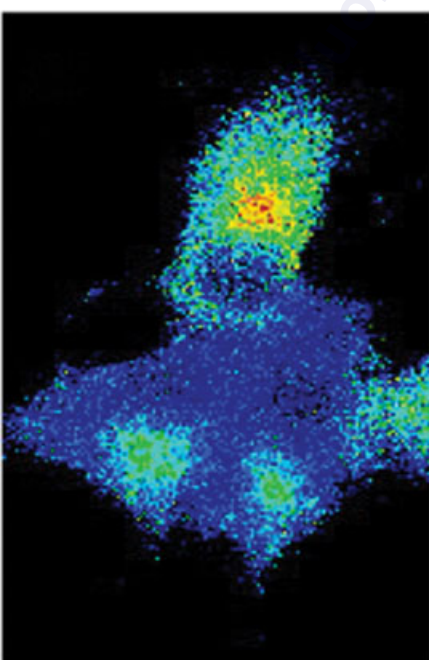
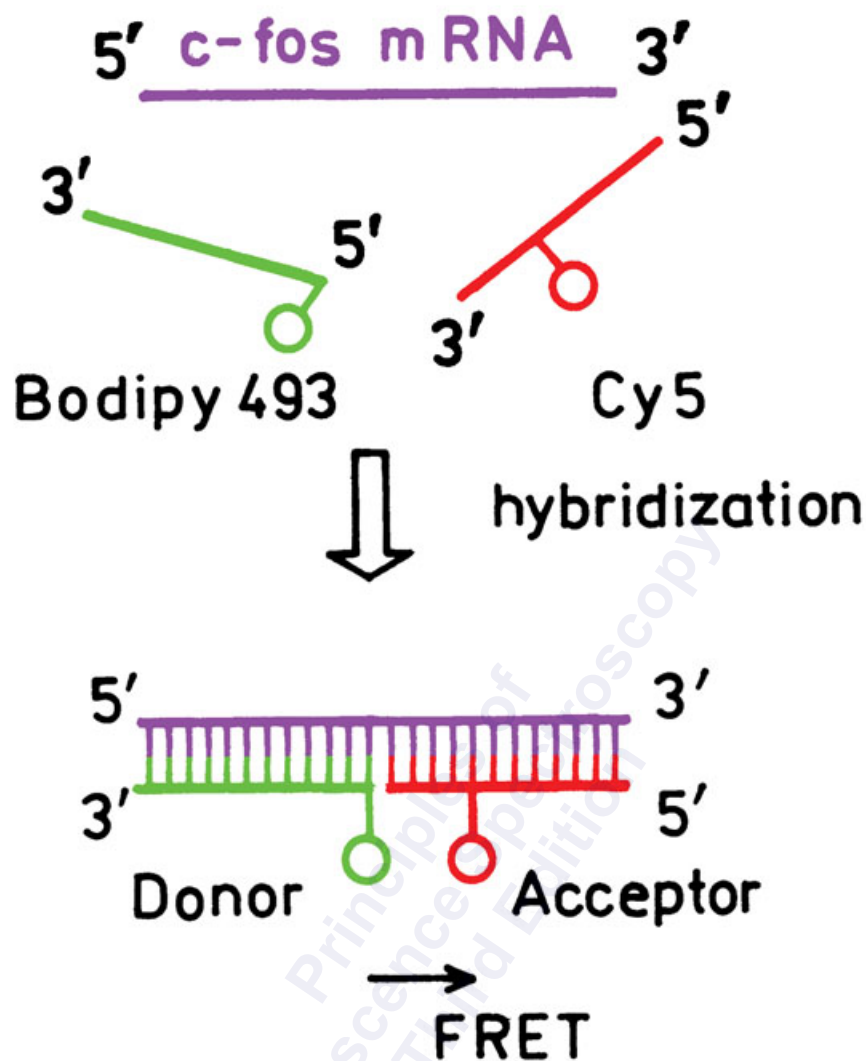


Acceptor:  
hexachlorofluorescein



FLUORESCENCE  
INTENSITY





2.0

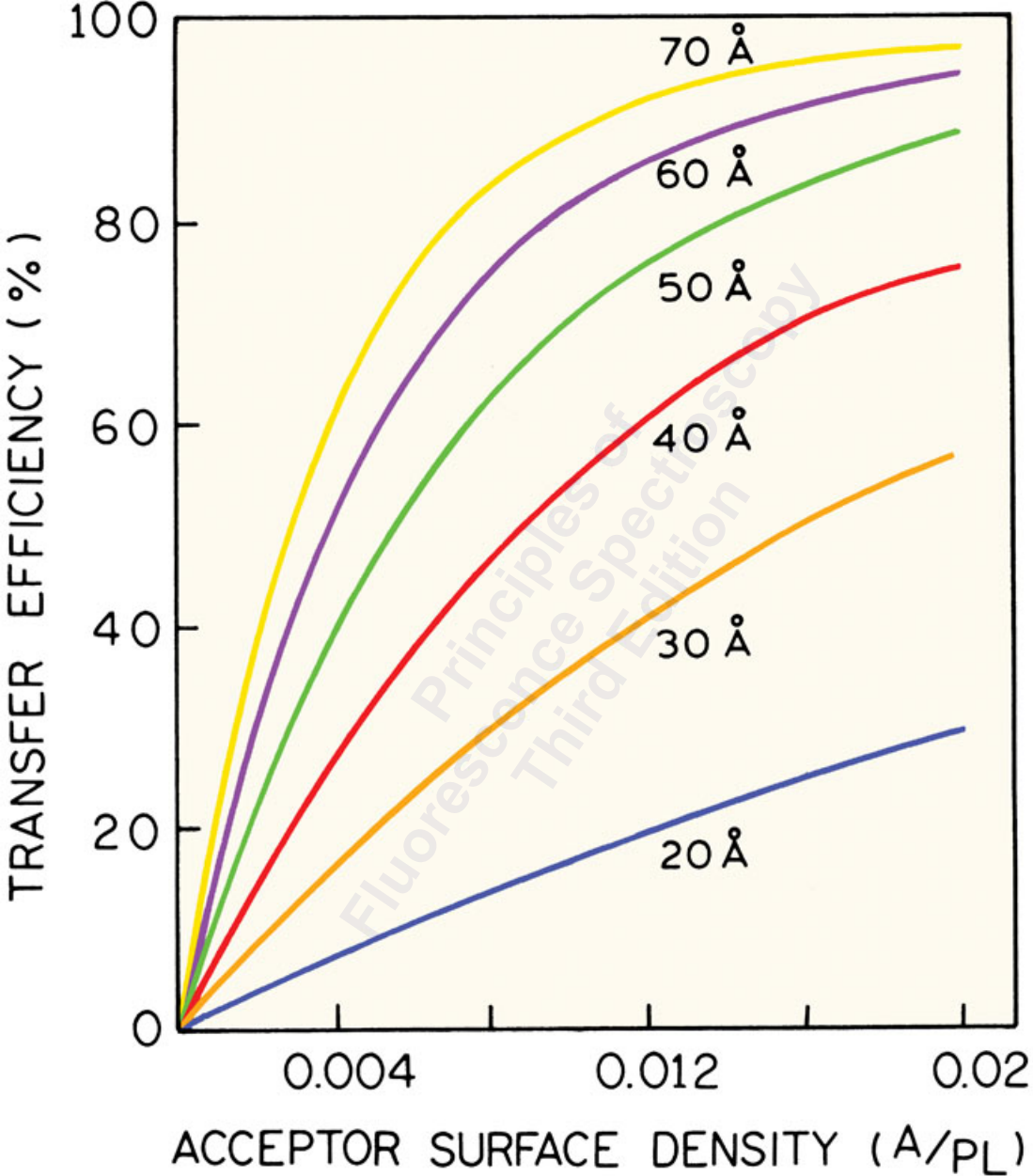
1.5

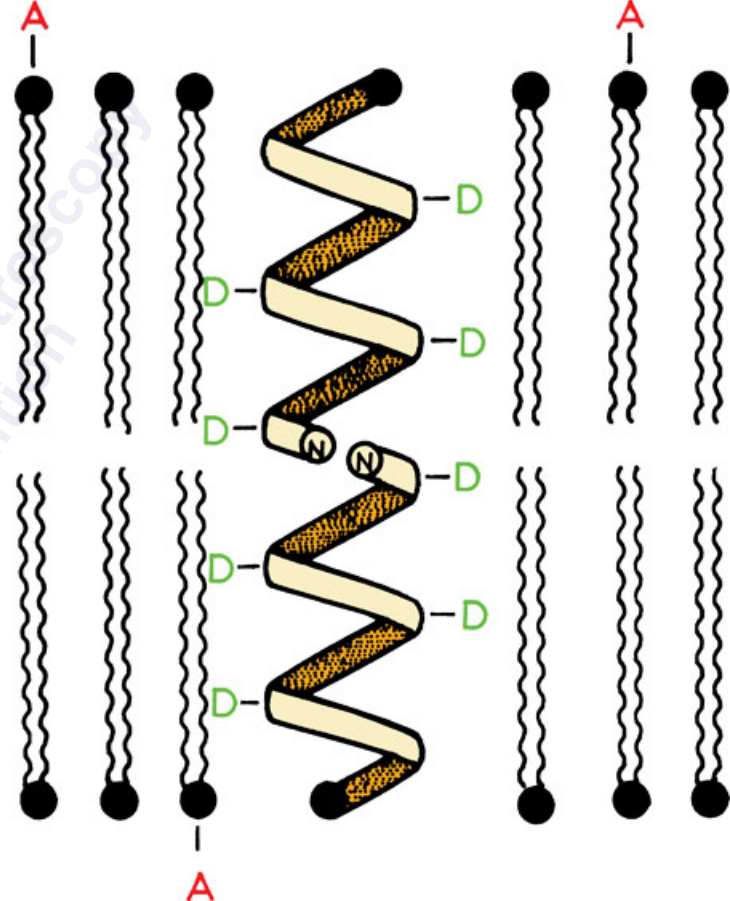
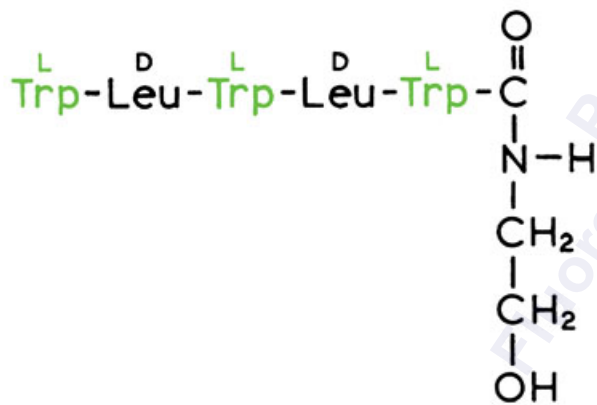
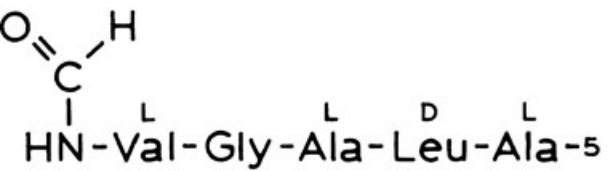
1.0

0.5

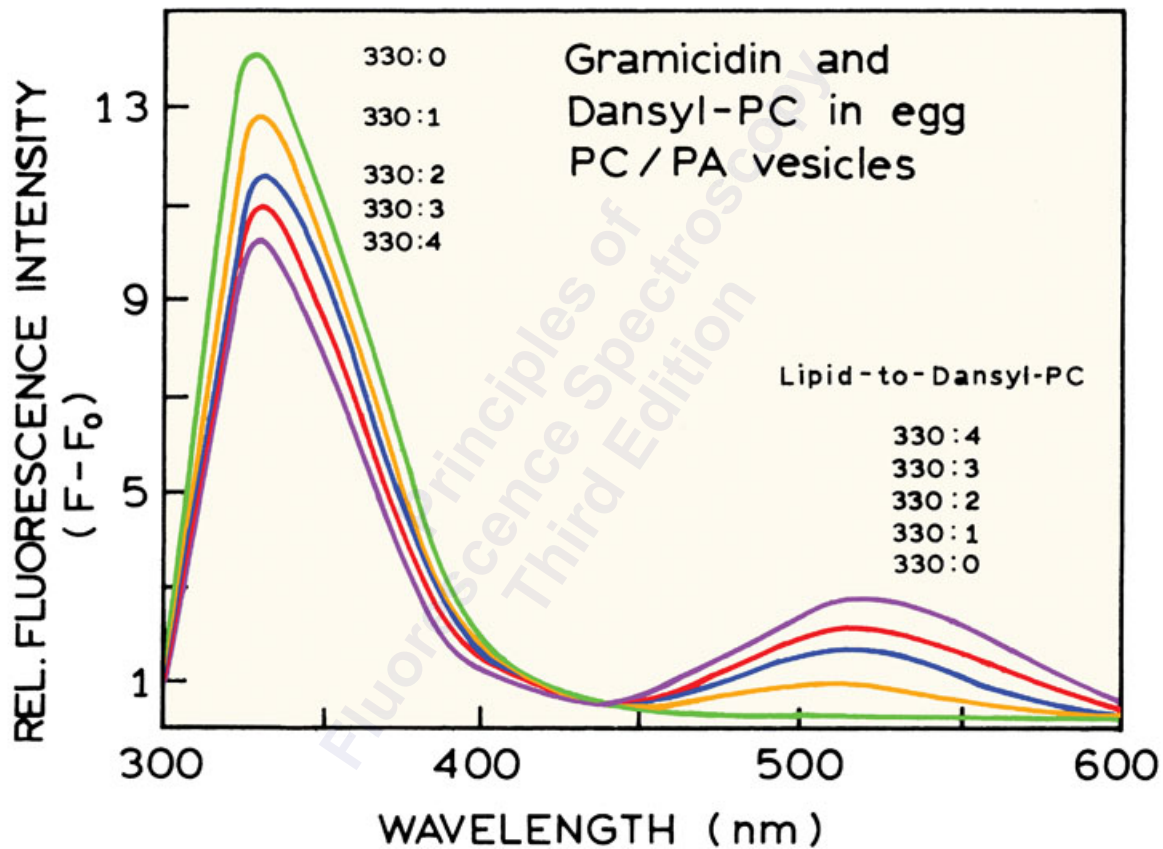
0.0

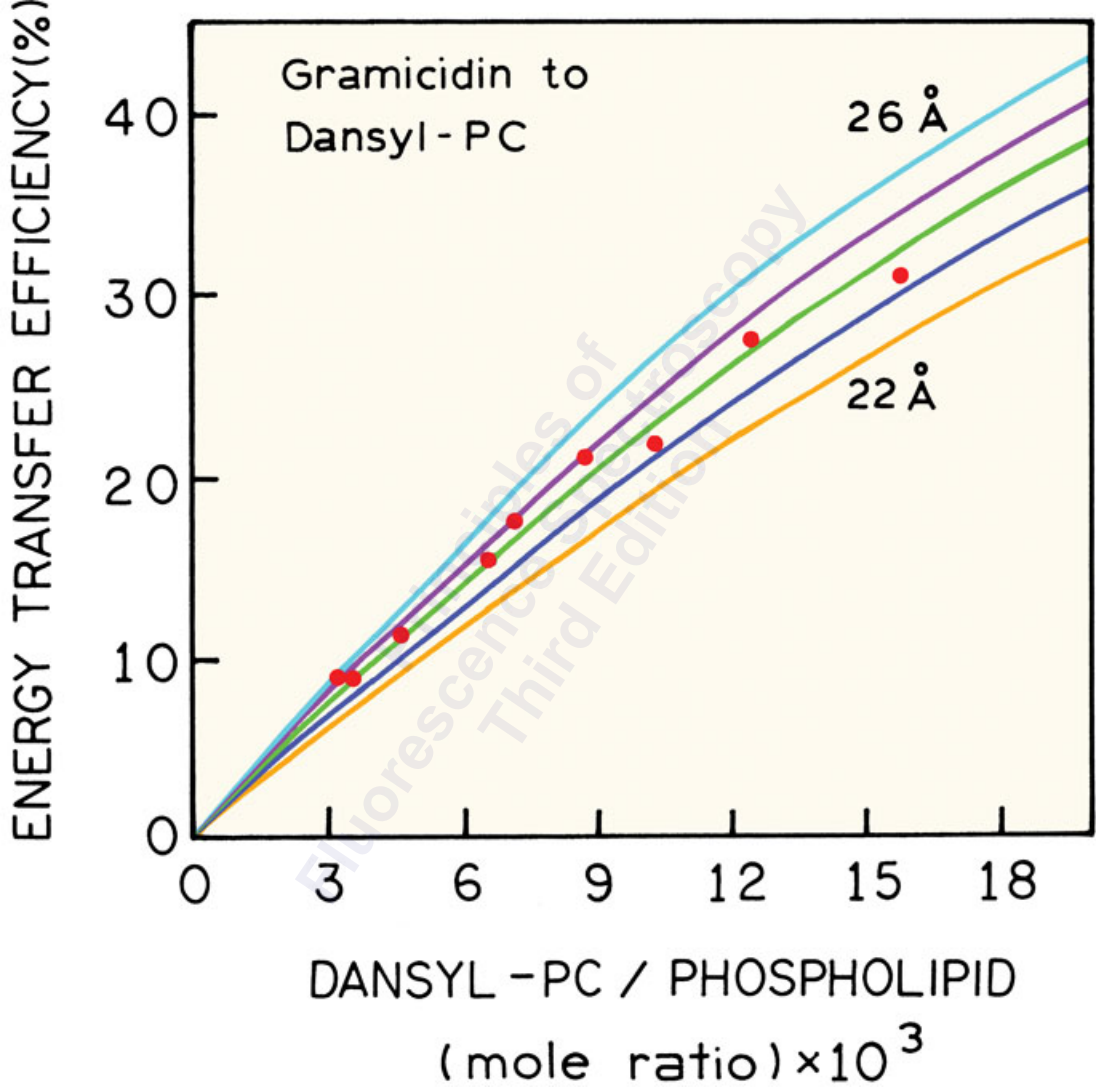


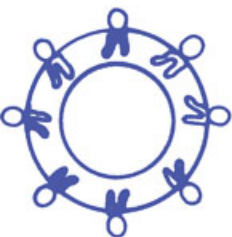




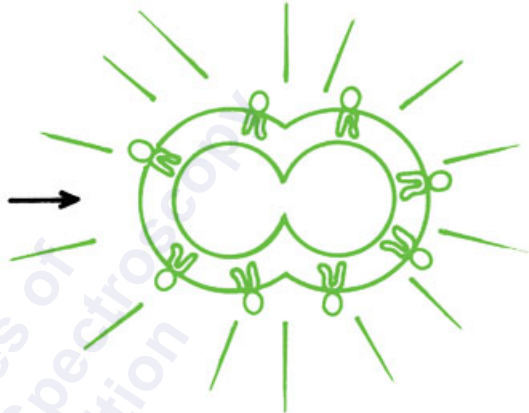








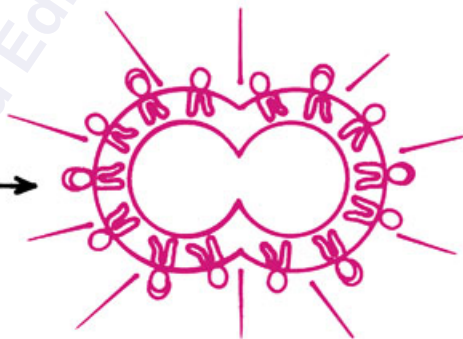
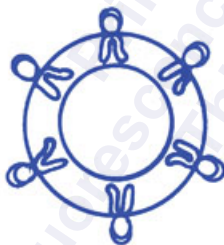
+



Self-Quenched

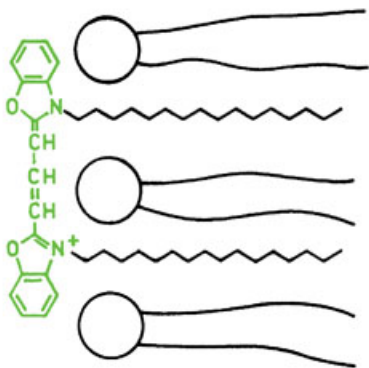


+

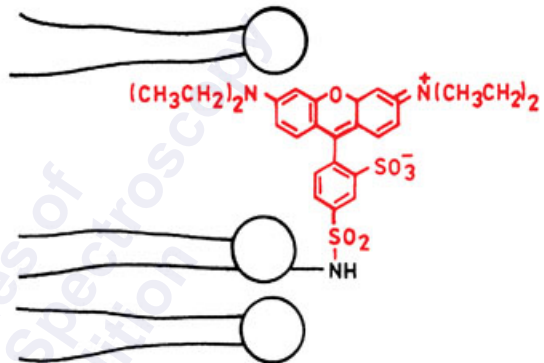


Donor

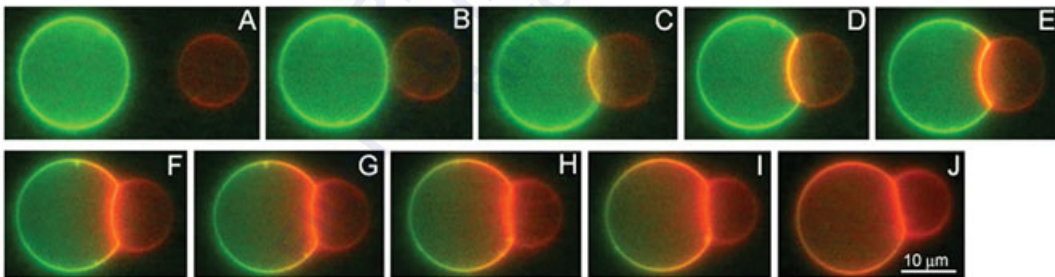
Acceptor

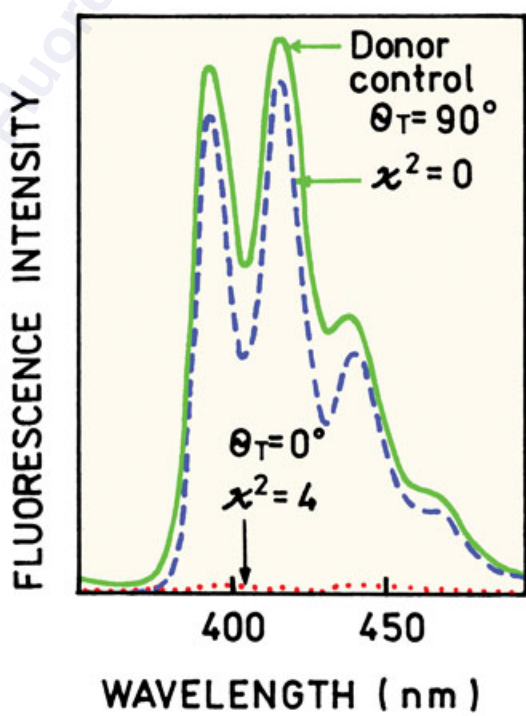
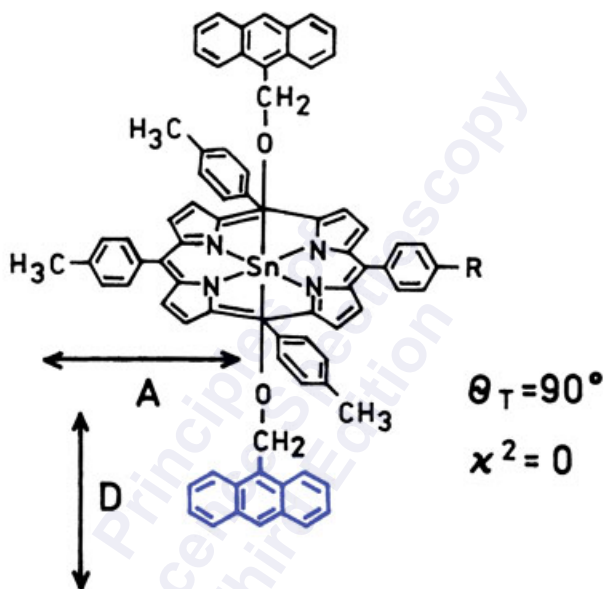
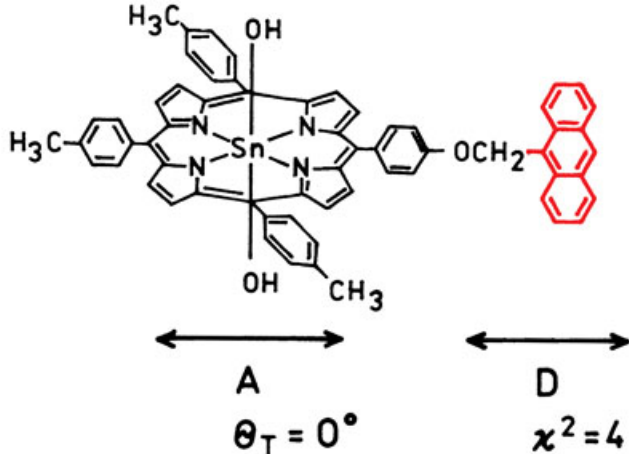


DiO



Rh-PE

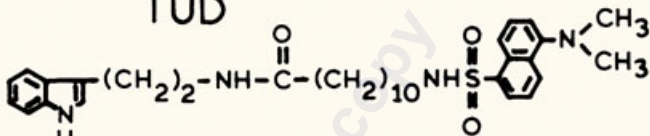




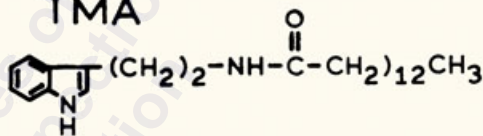
FLUORESCENCE INTENSITY

1.0

TUD



TMA



TMA

TUD

DUA

0

300

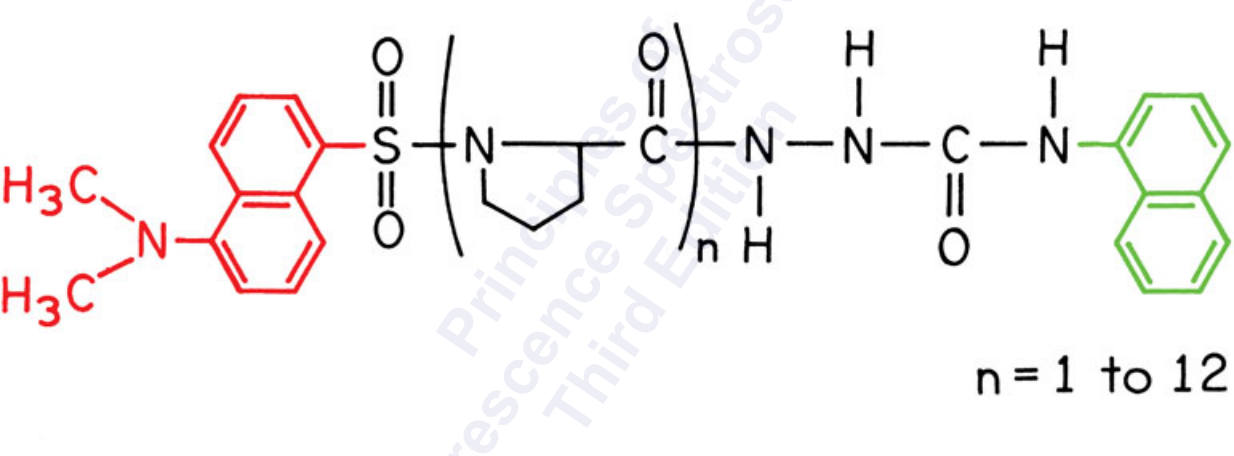
400

500

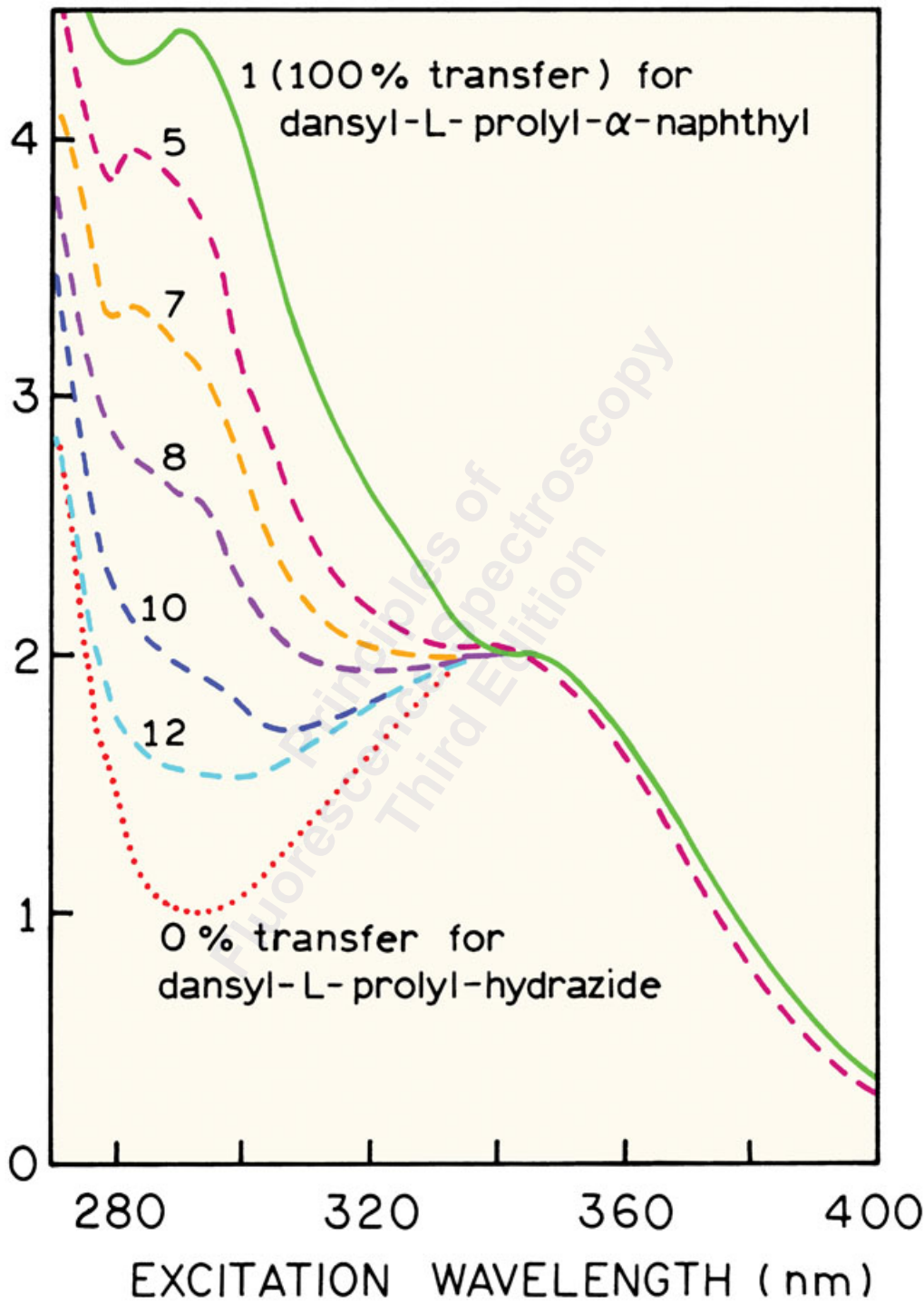
600

WAVELENGTH (nm)

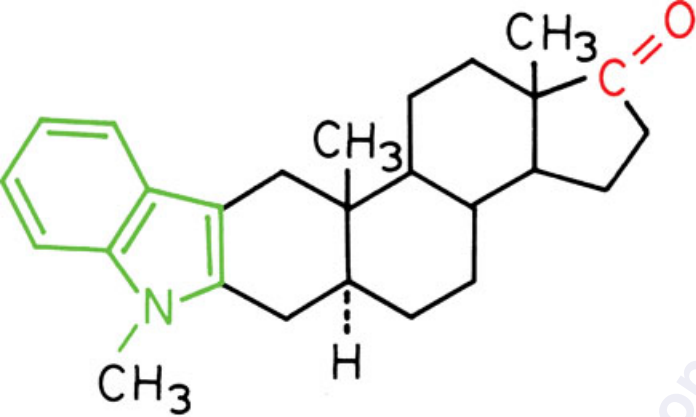
Fluorescence Spectroscopy  
Third Edition



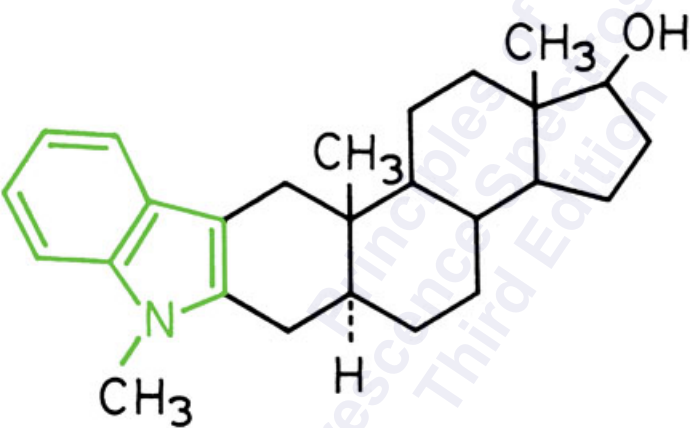
FLUORESCENCE INTENSITY



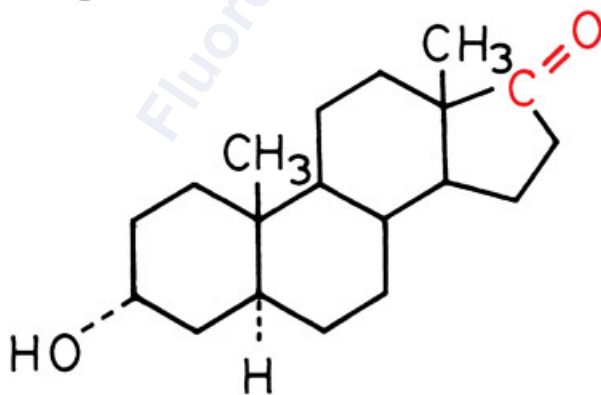




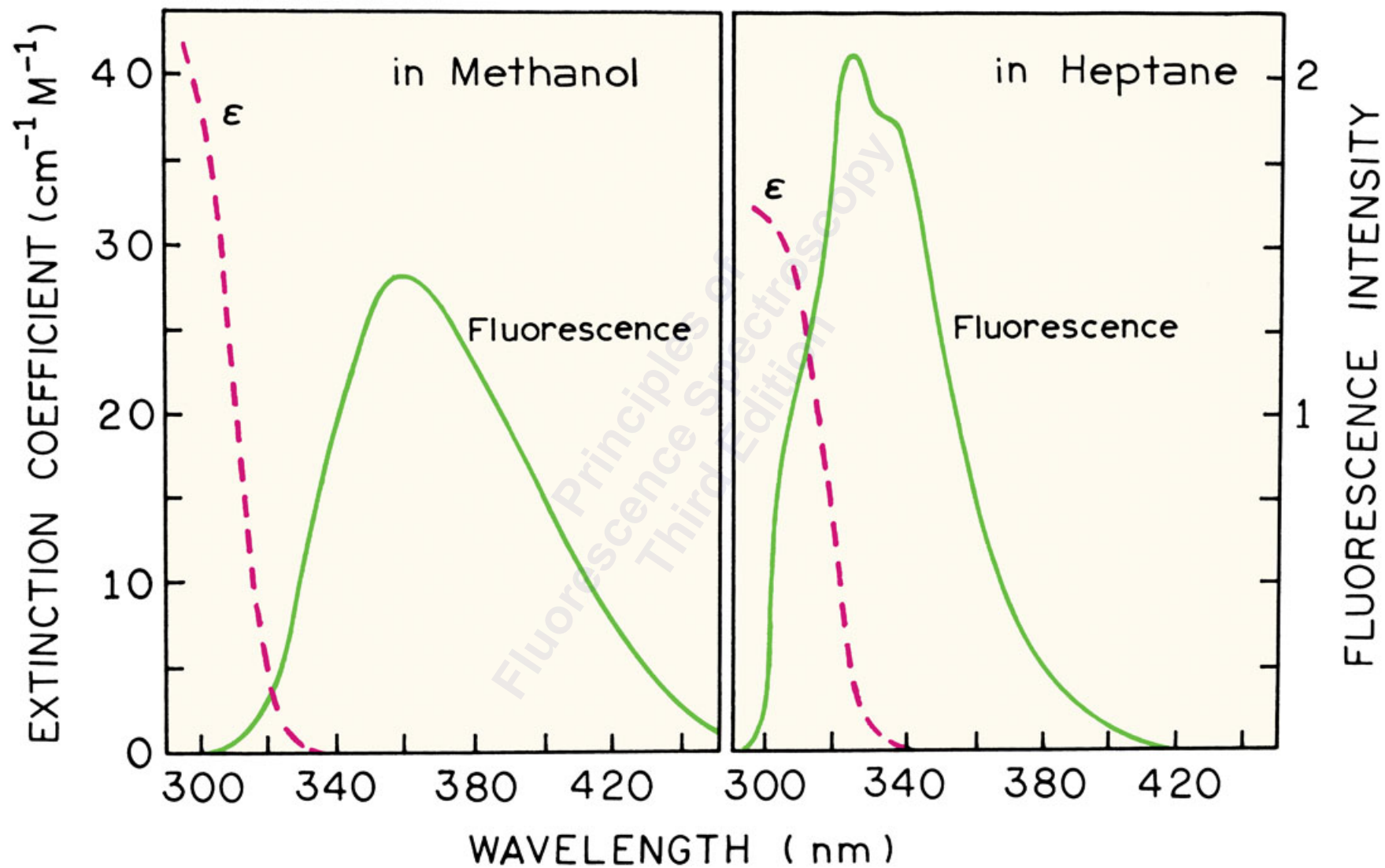
I

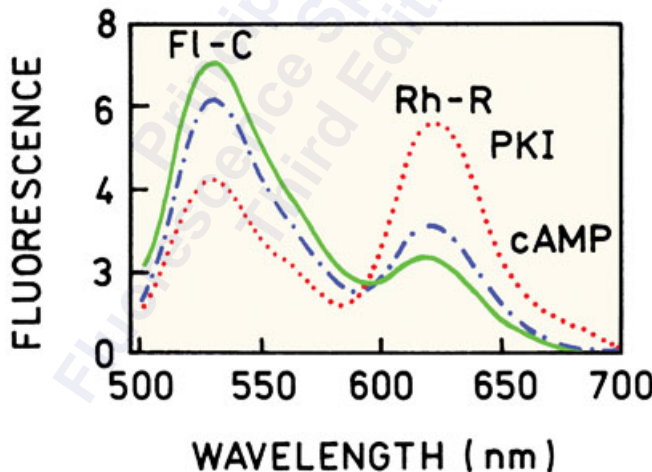
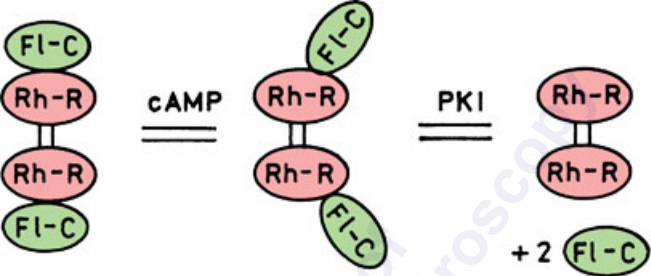


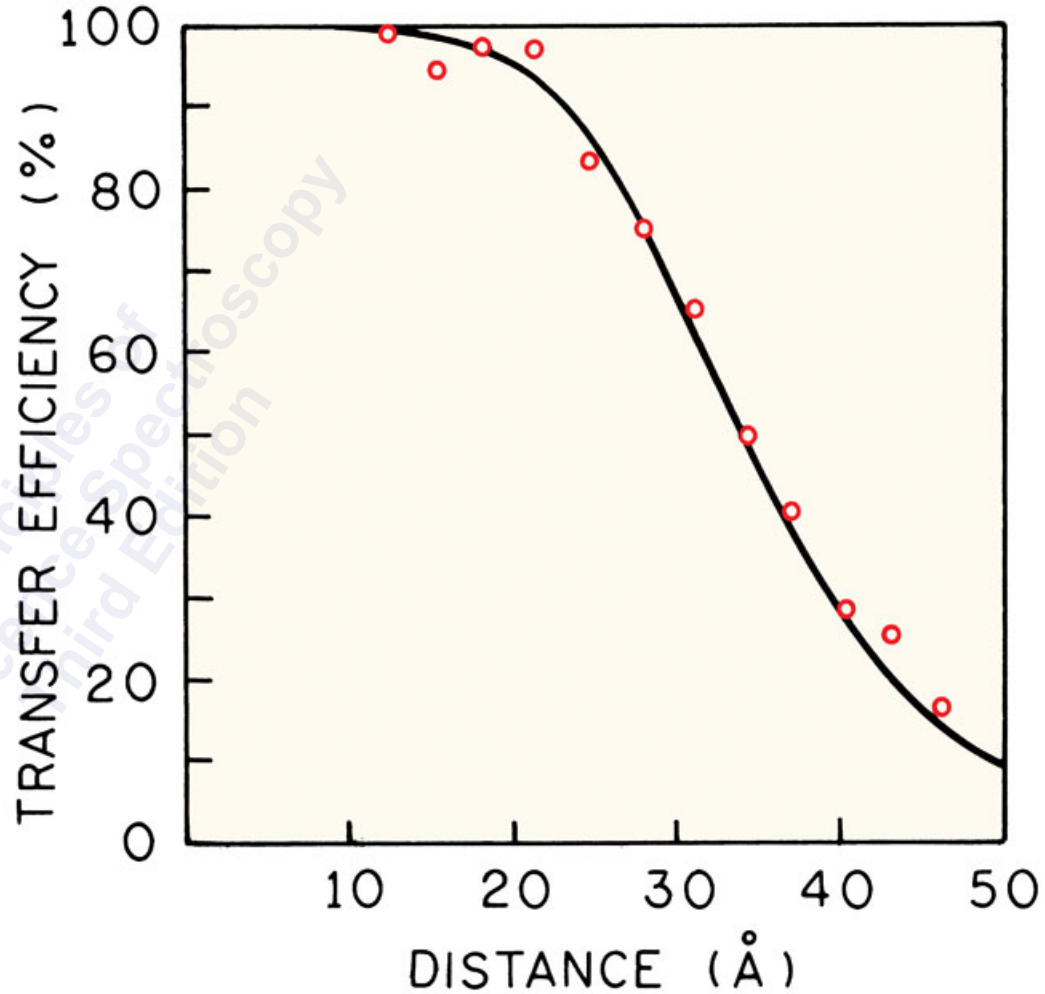
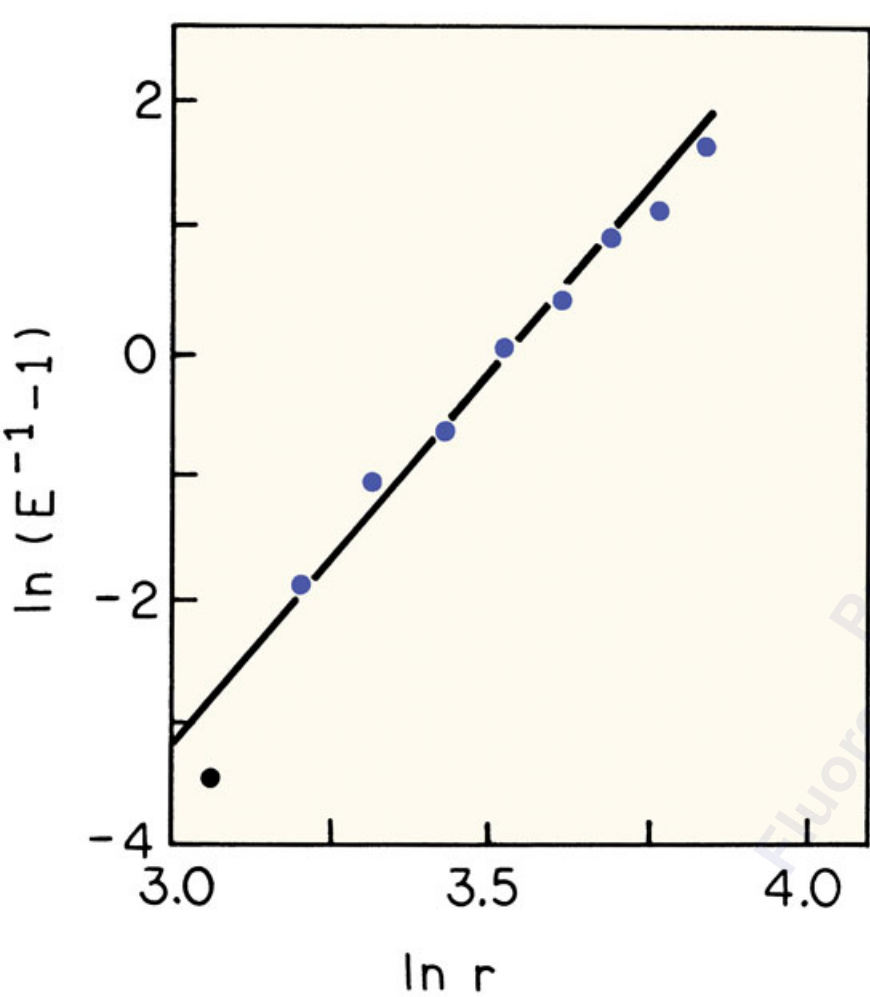
II

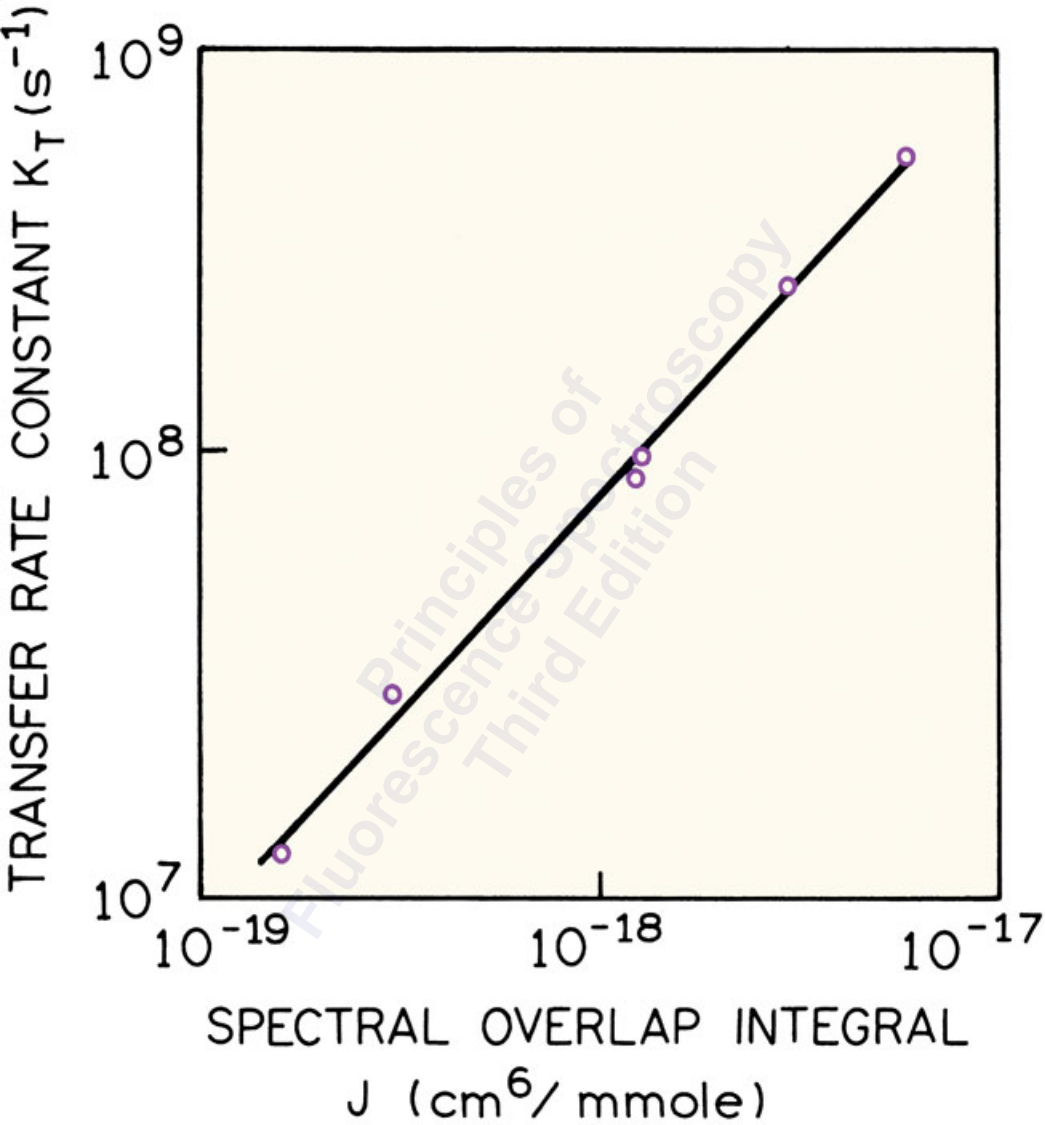


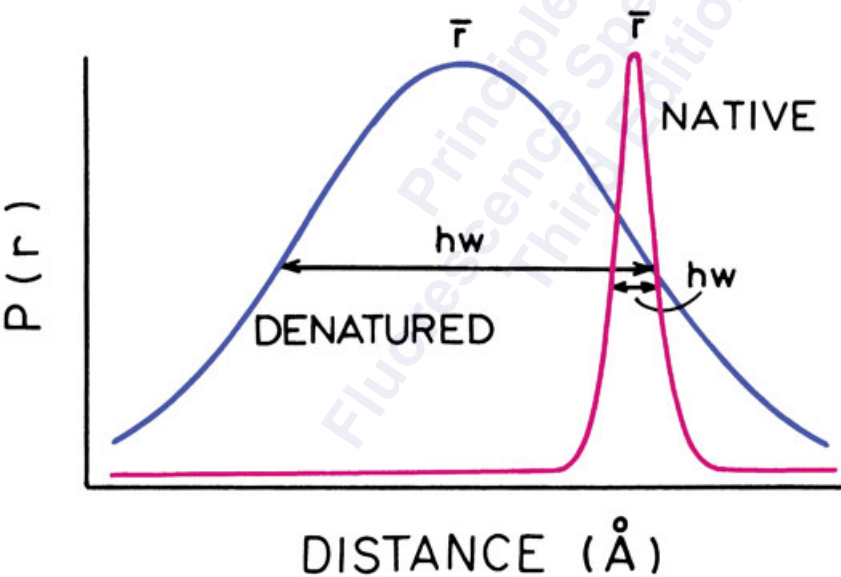
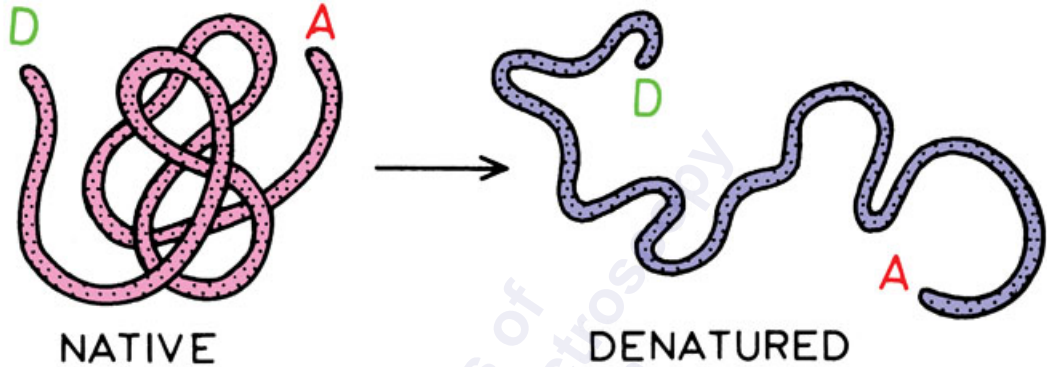
III

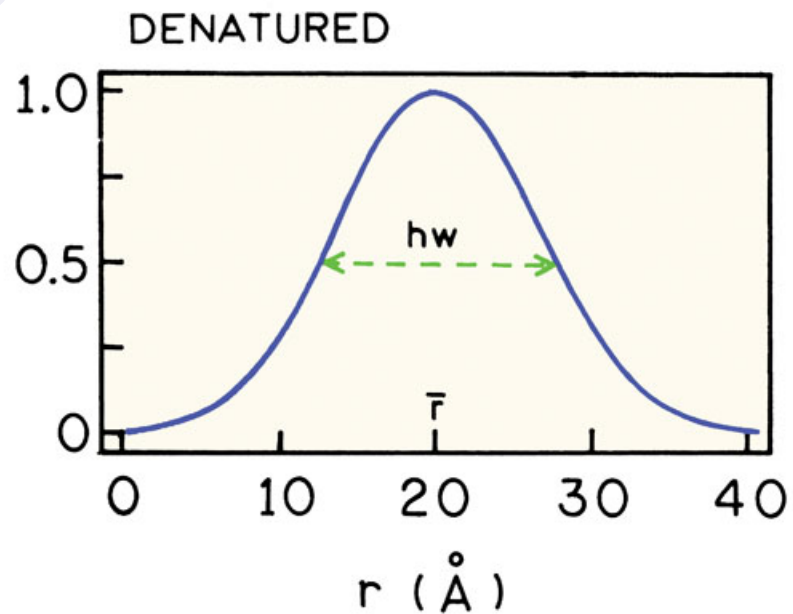
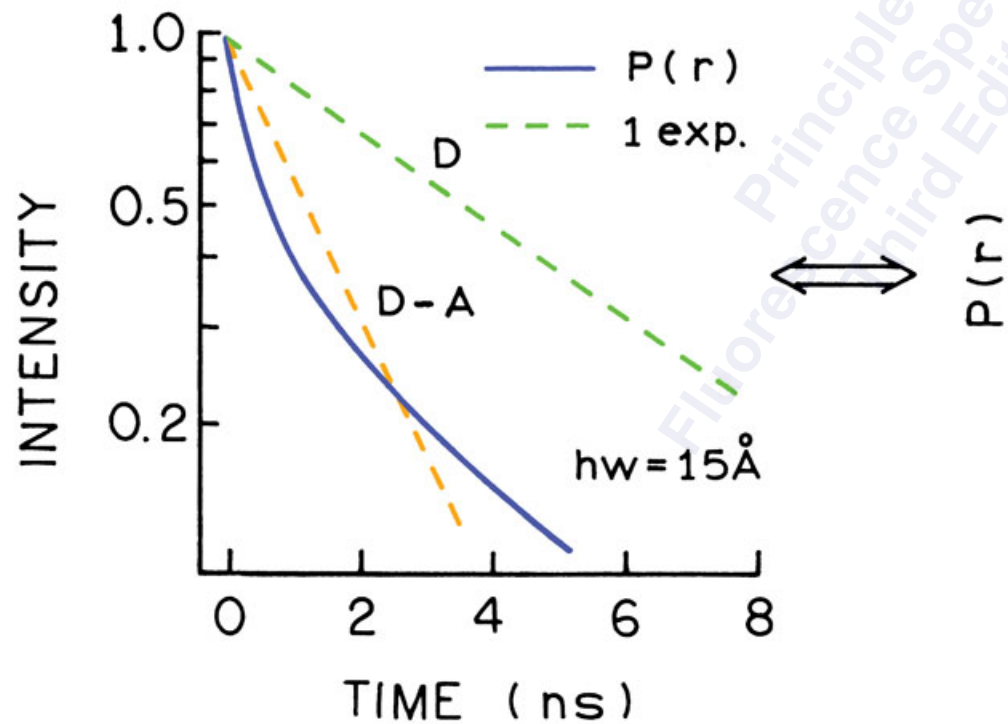
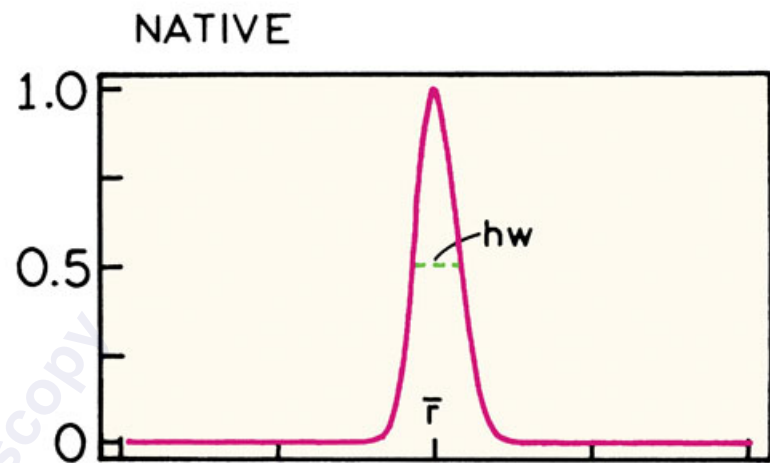
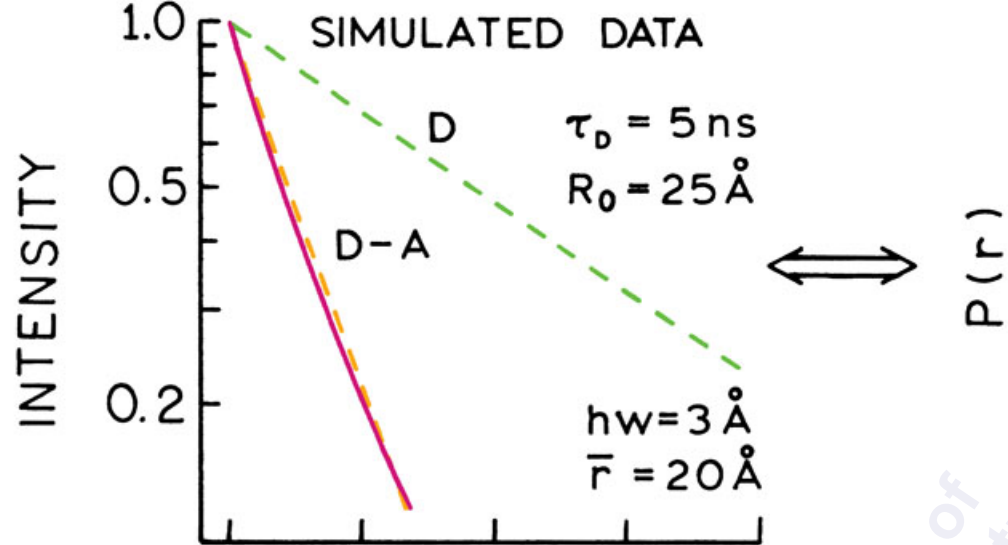




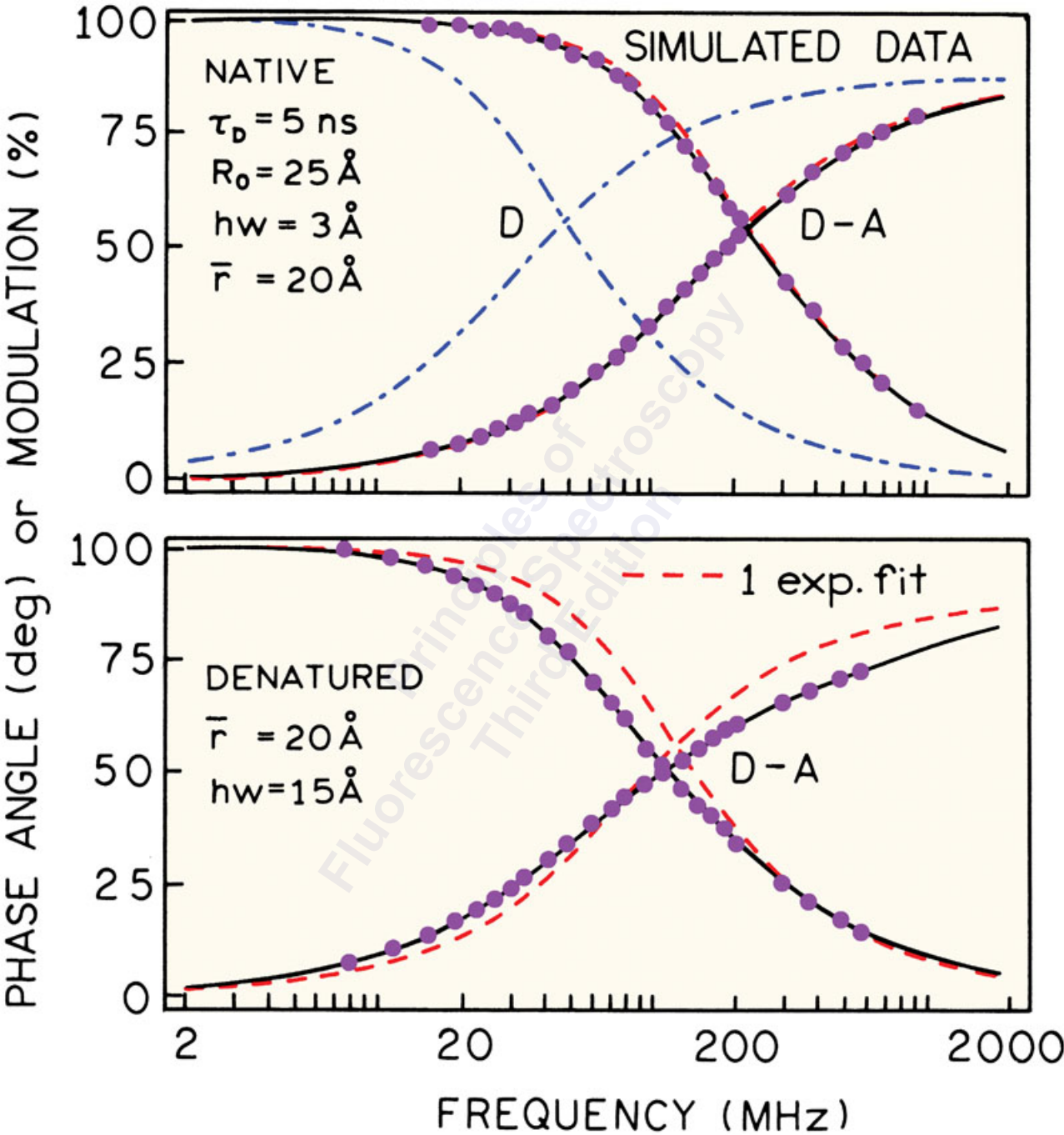




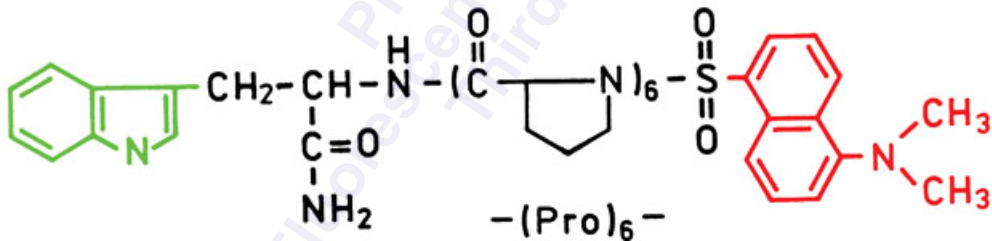
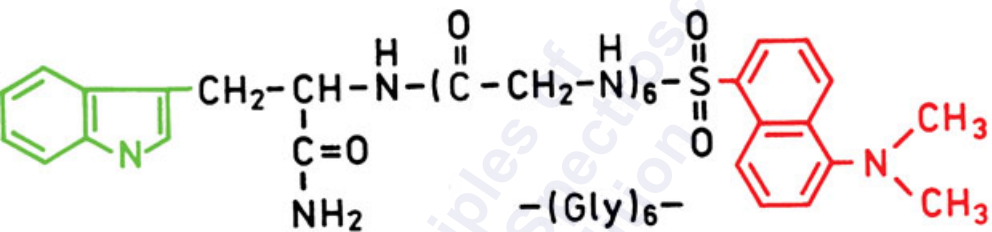


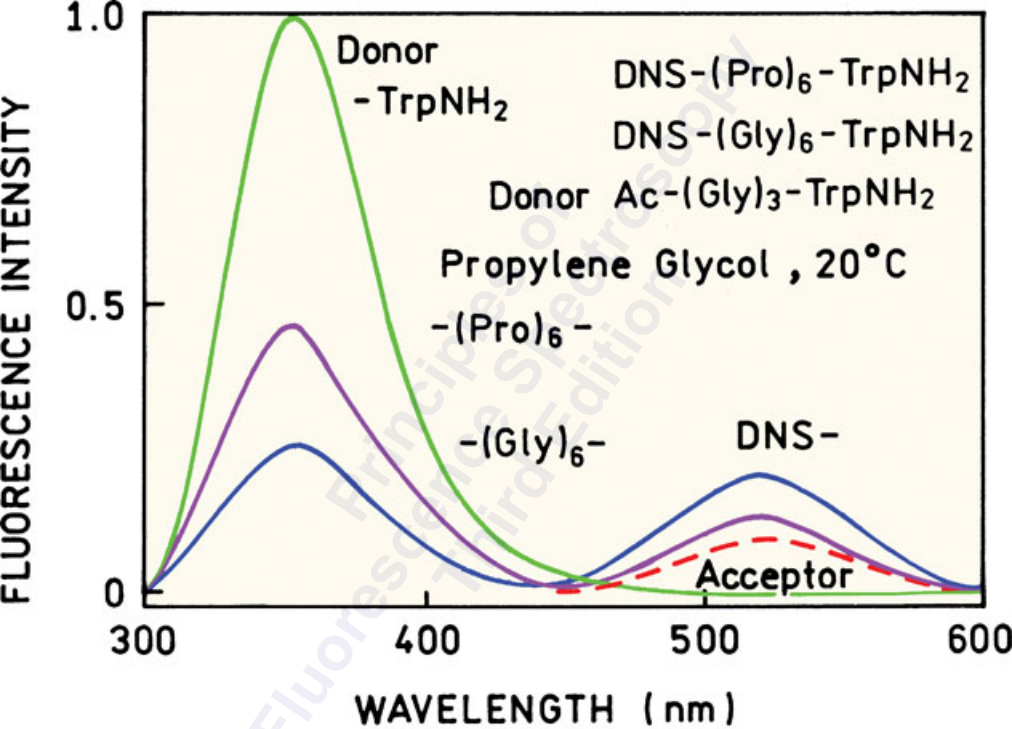


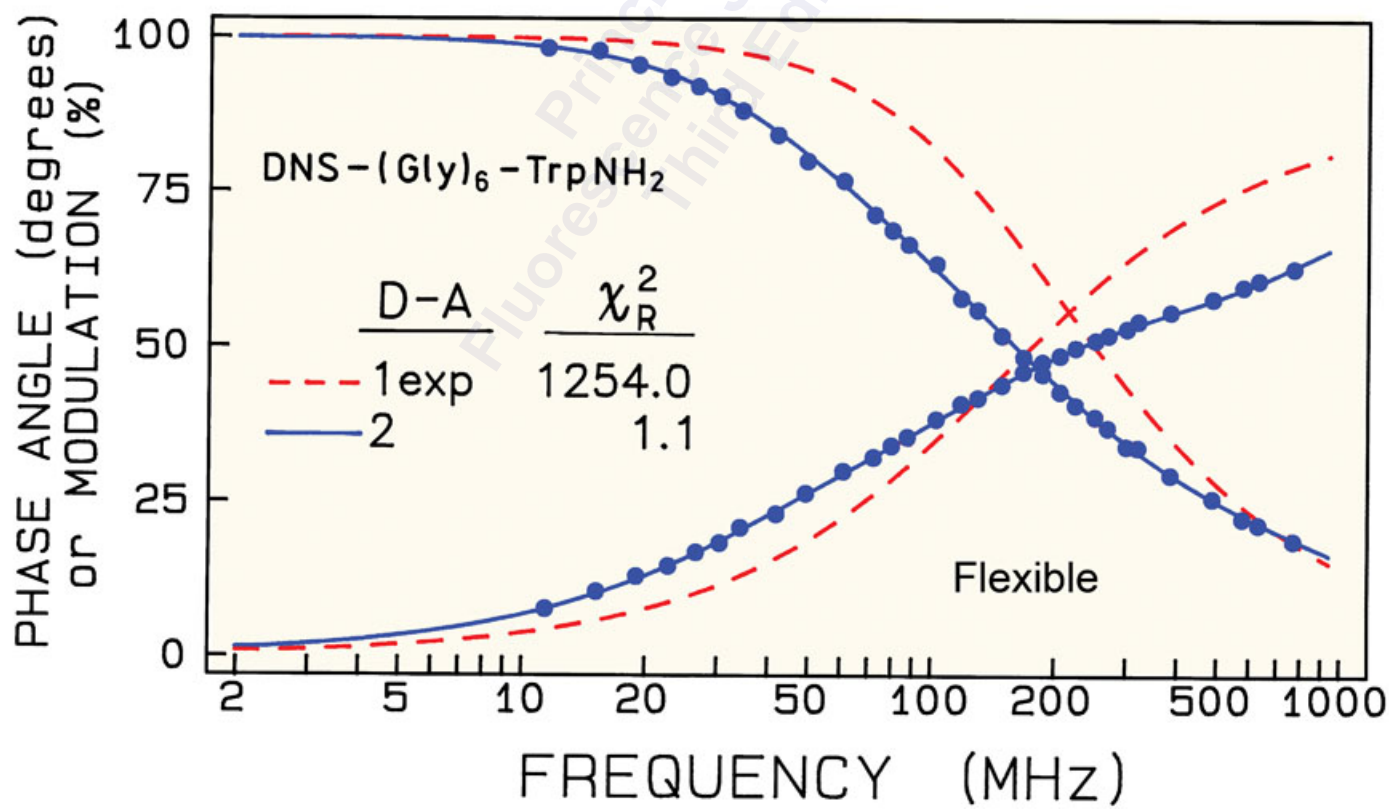
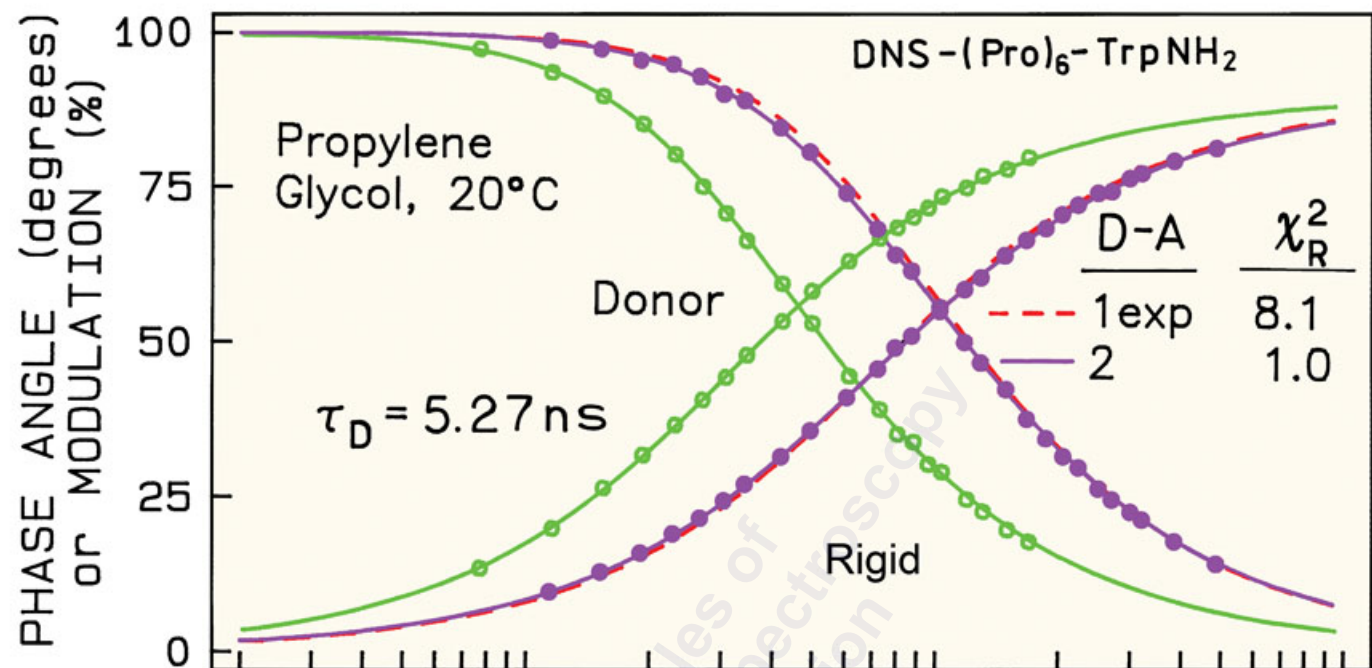


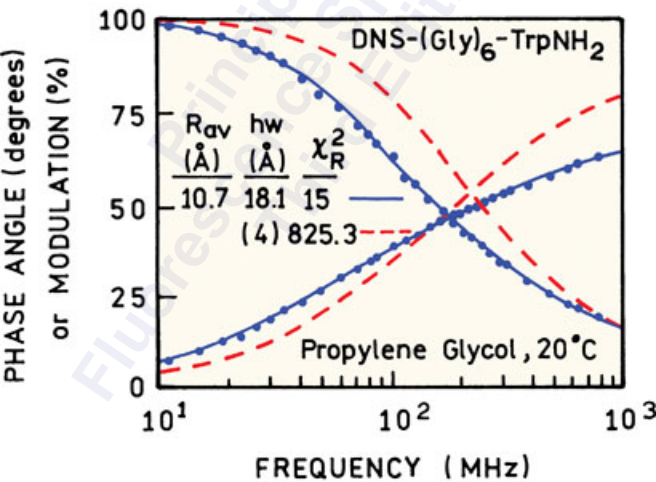
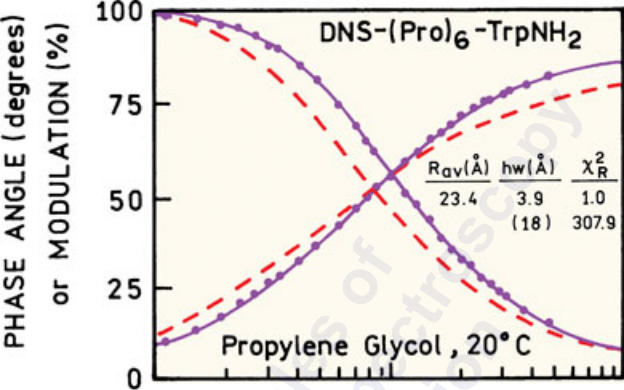


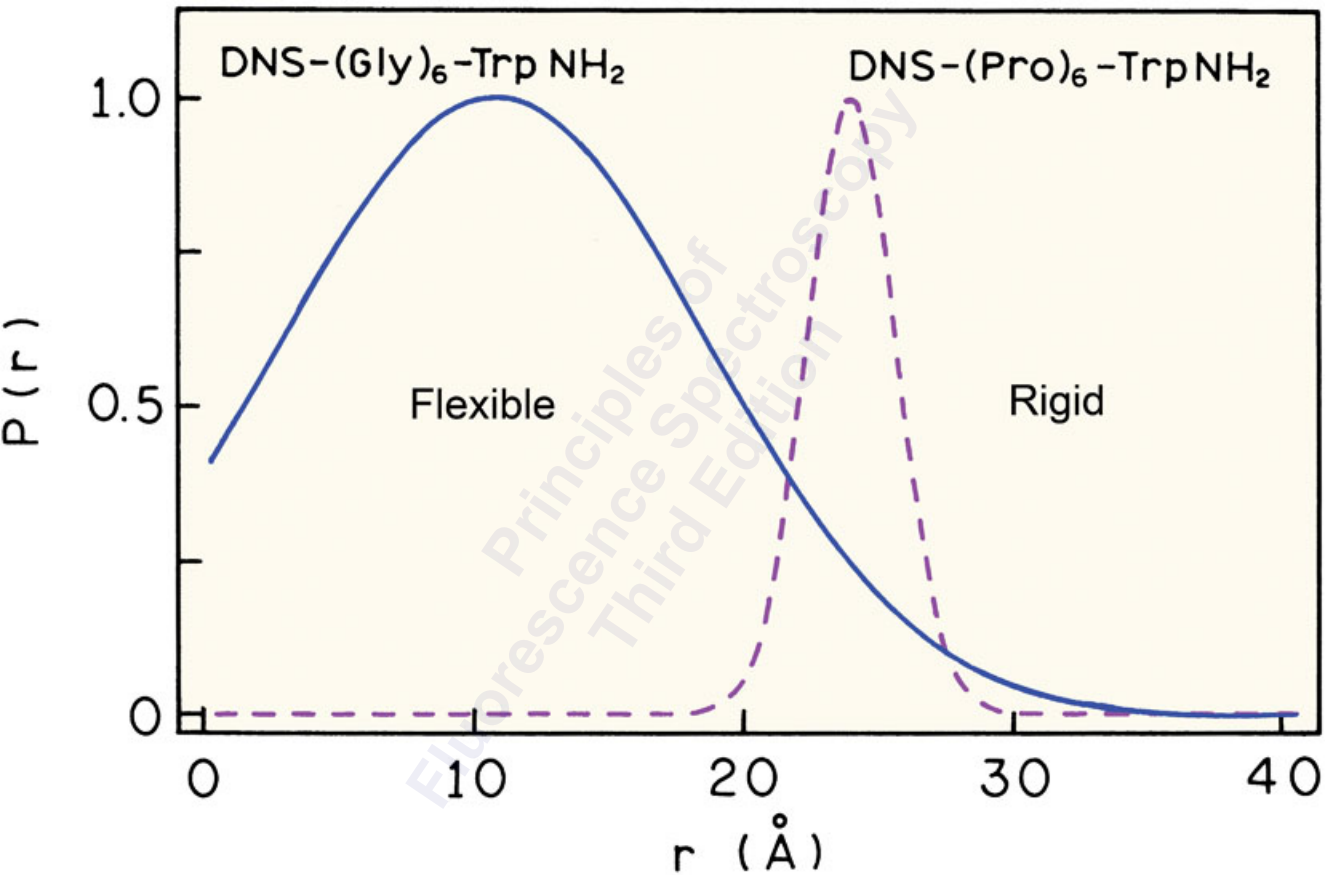


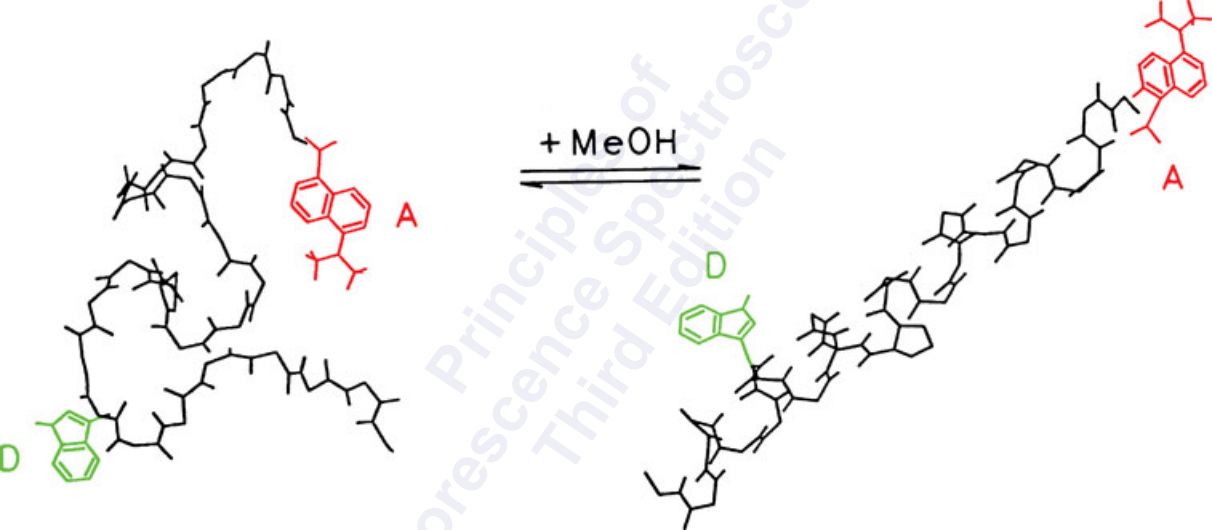


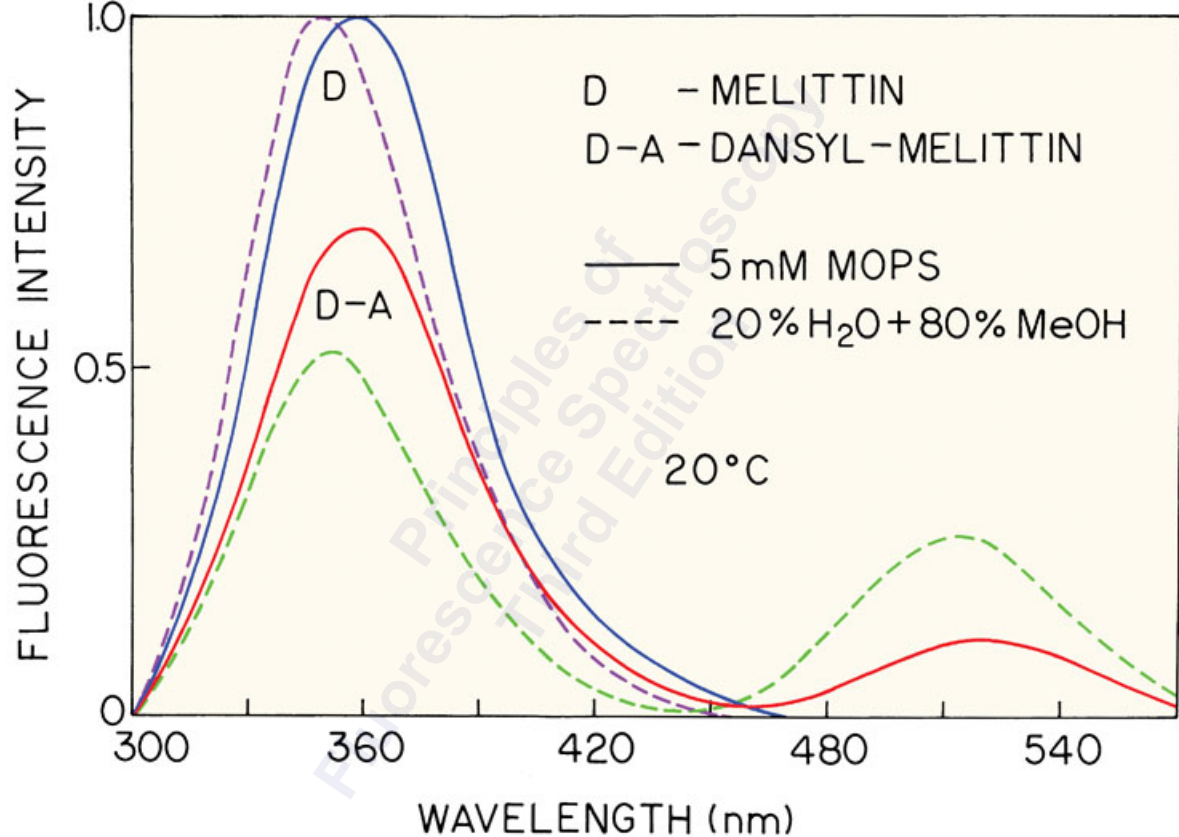


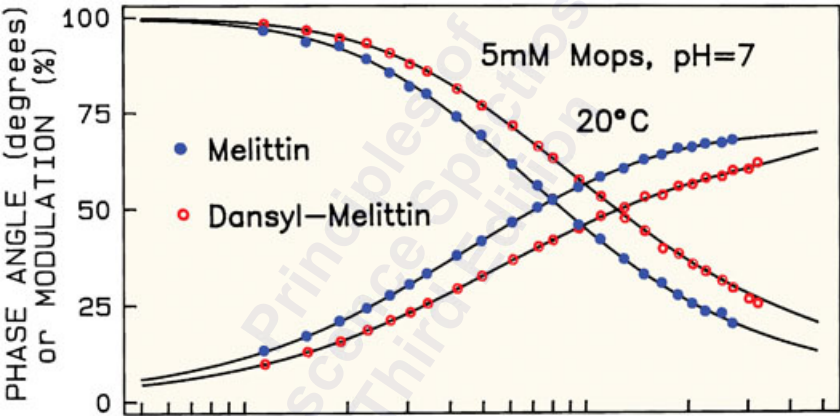






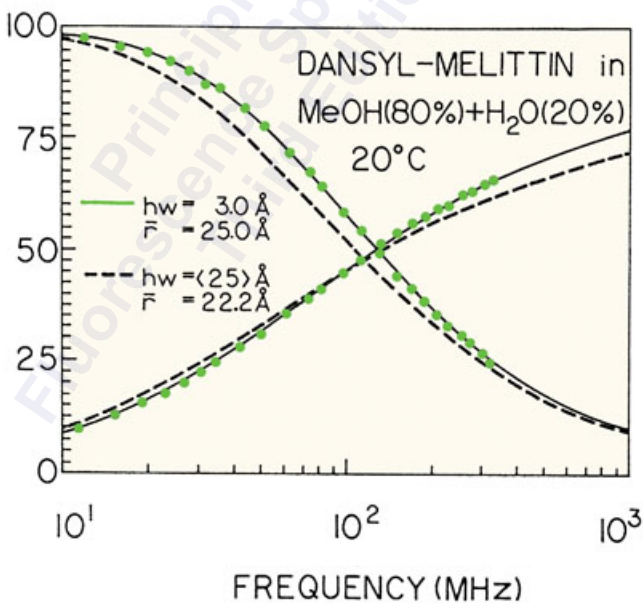
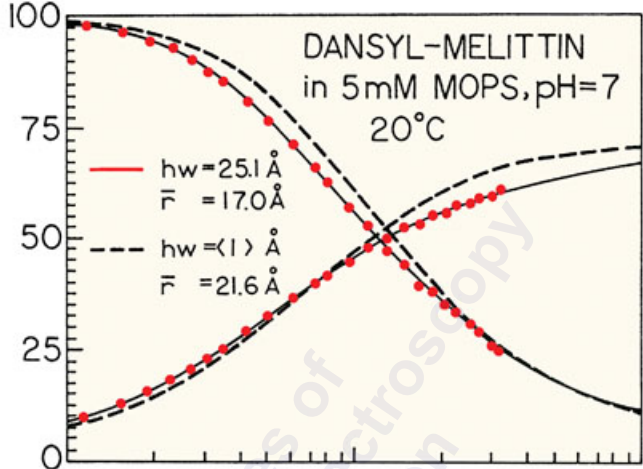


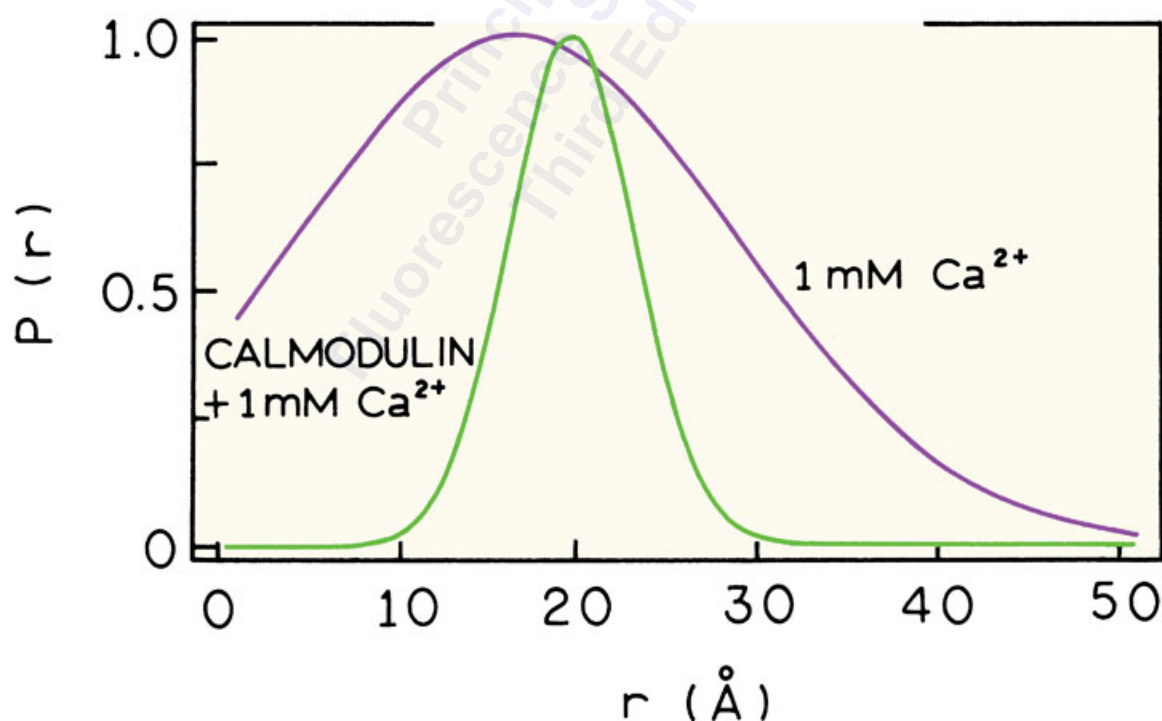
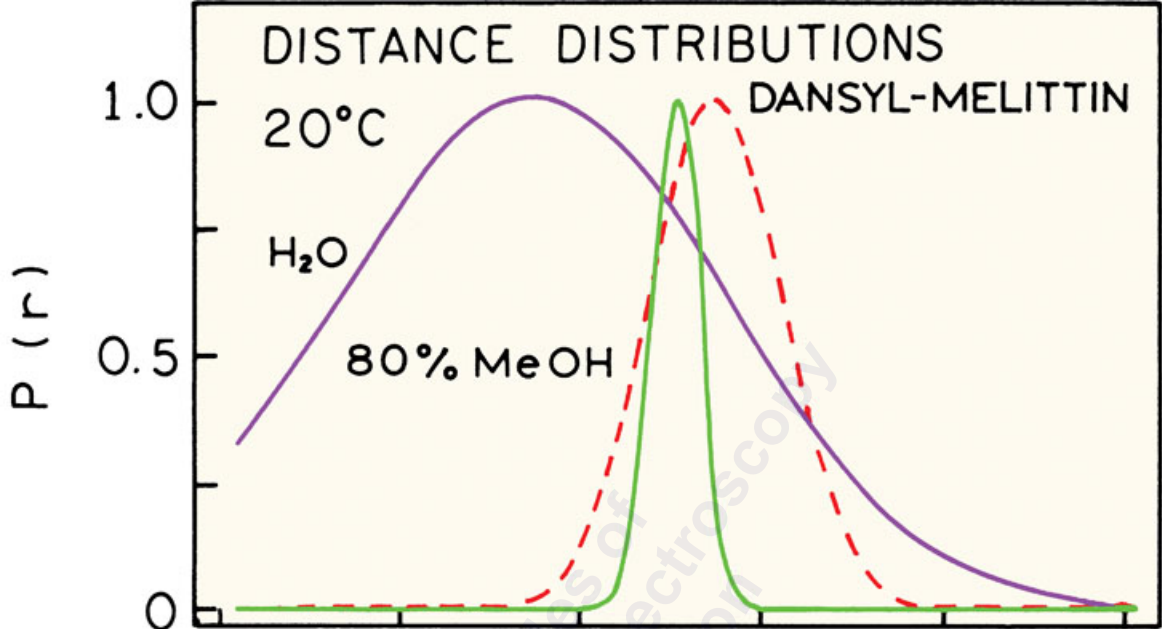






PHASE ANGLE (degrees)  
or MODULATION (%)

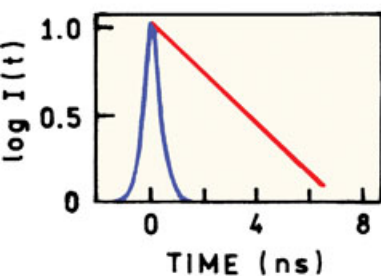




## TIME - DOMAIN

## THEORY

## FREQUENCY - DOMAIN



$$k_T(r) = \frac{1}{\tau_0} \left( \frac{R_0}{r} \right)^6$$



Observables  
Counts / Channel

$$I_{DA} = \int_0^{\infty} P(r) I_{DA}(r, t) dr$$

Observables  
 $\phi_\omega$  and  $m_\omega$

Convolution  
Integral

$$R(t) = \int_0^t L(t') F(t-t') dt'$$

Fourier  
Transforms

$$\tan \phi_\omega = N_\omega / D_\omega$$

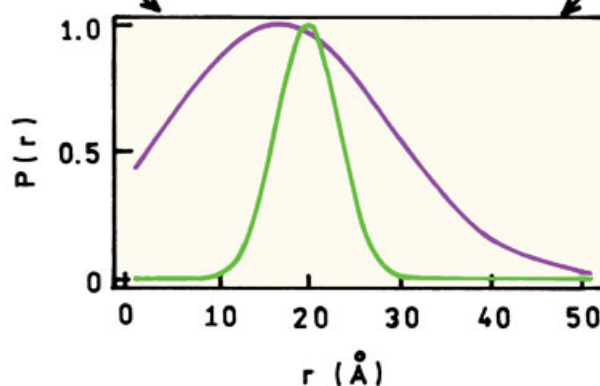
$$m_\omega = [N_\omega^2 + D_\omega^2]^{1/2}$$

Least Squares  
Analysis

$$\chi_R^2$$

Least Squares  
Analysis

$$\chi_R^2$$

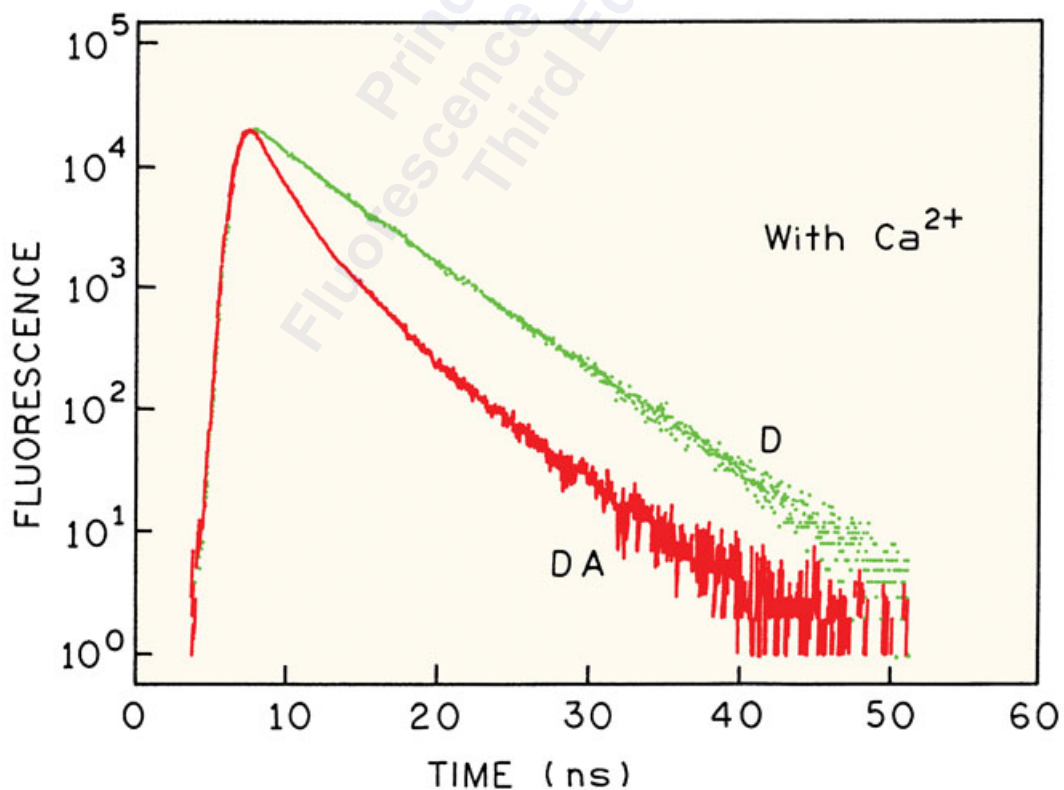
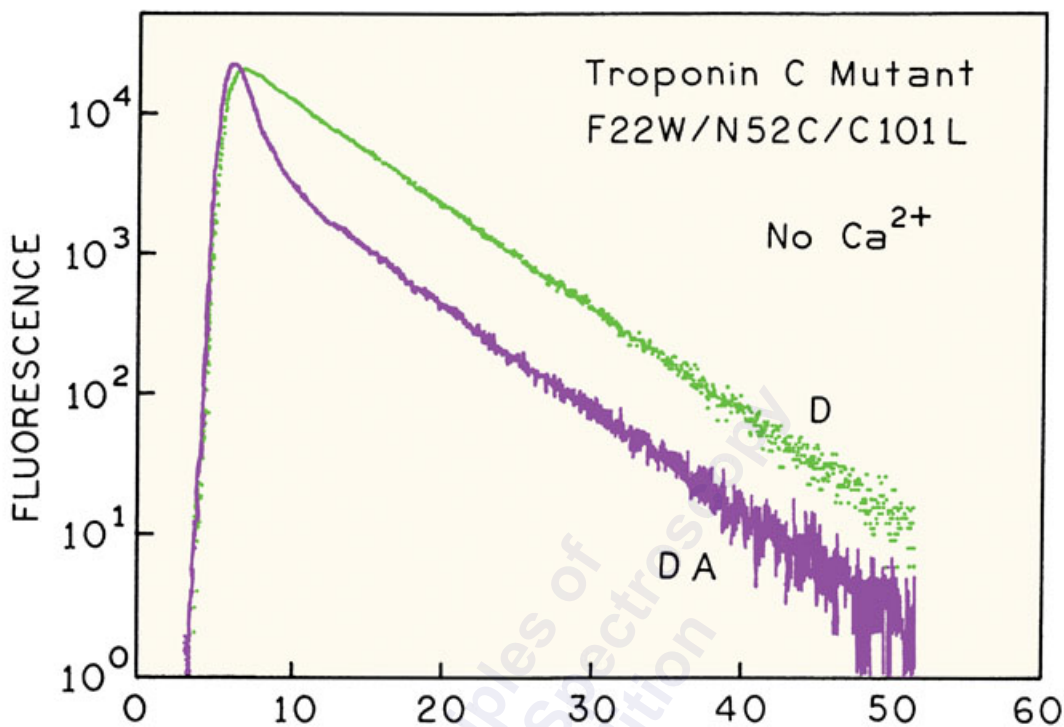


IAEDANS-52

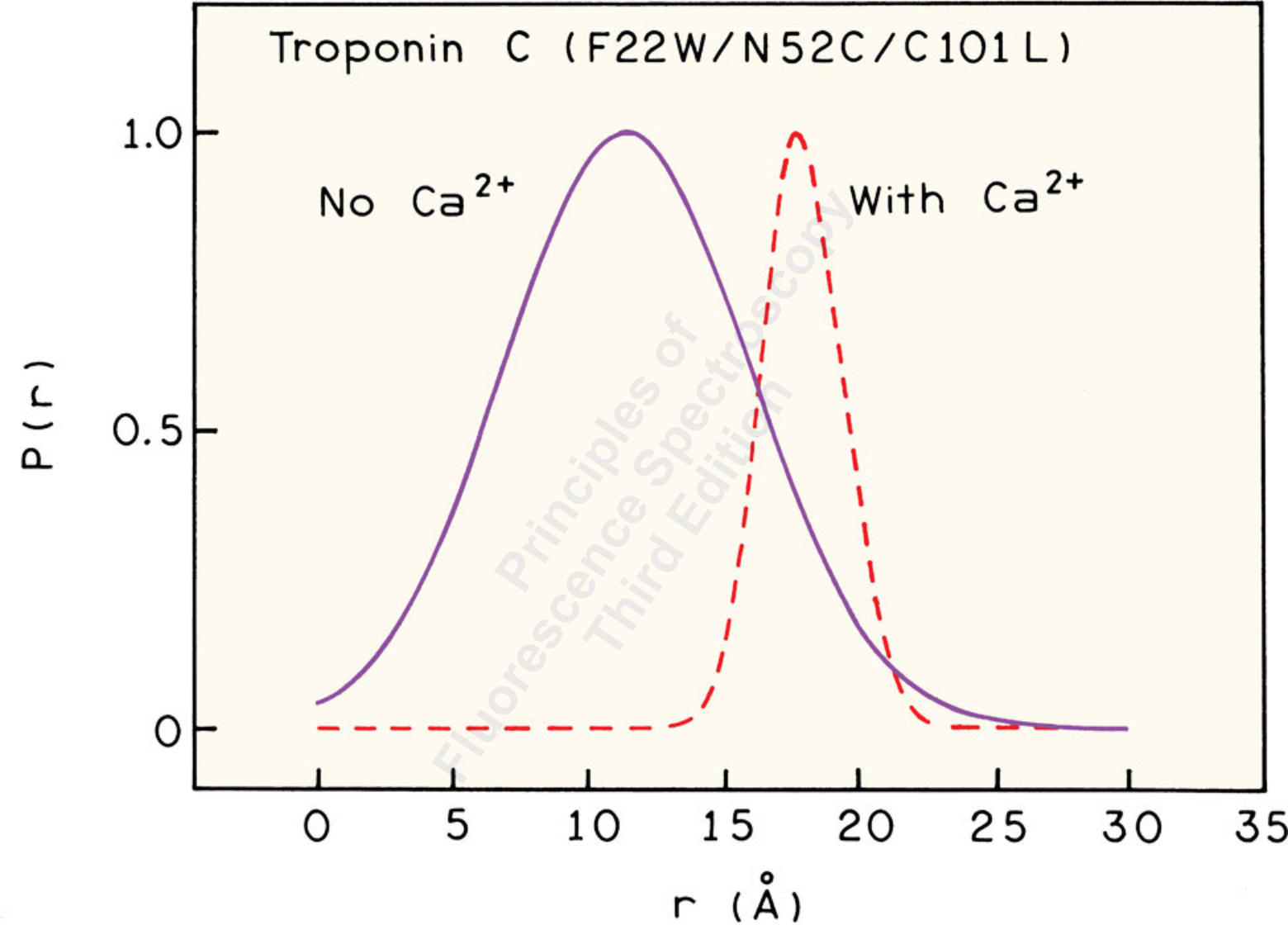
Trp-22

Principles of Fluorescence Spectroscopy, 4th Edition

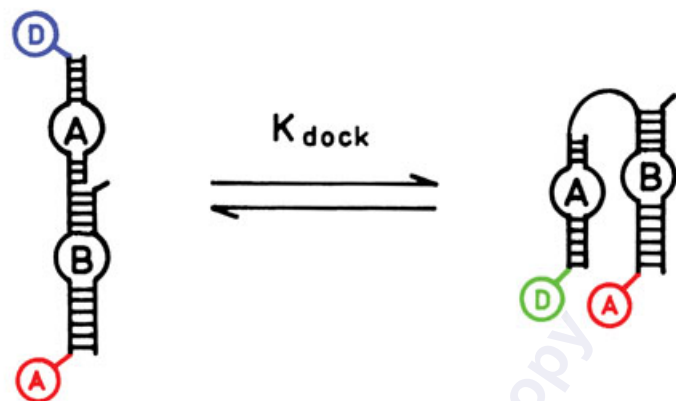




# Troponin C (F22W/N52C/C101L)

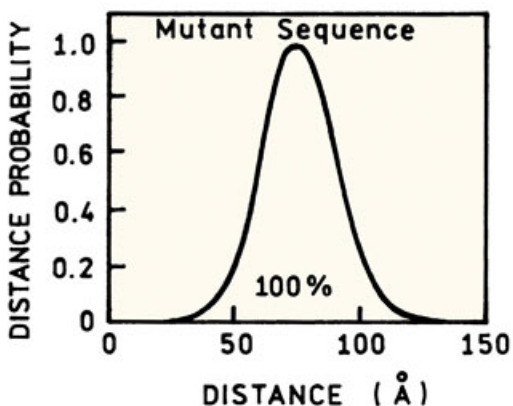
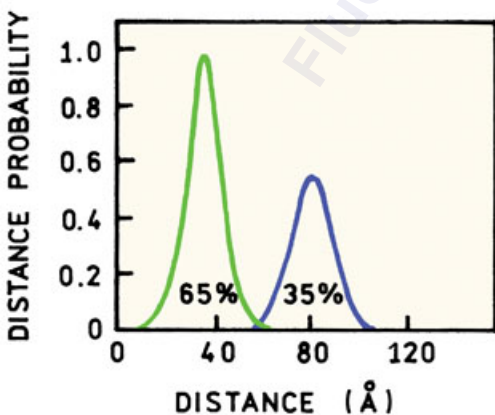
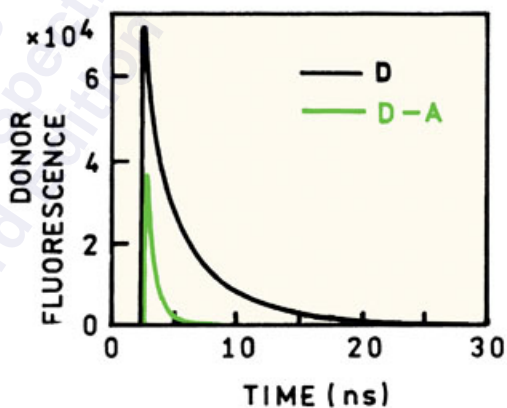
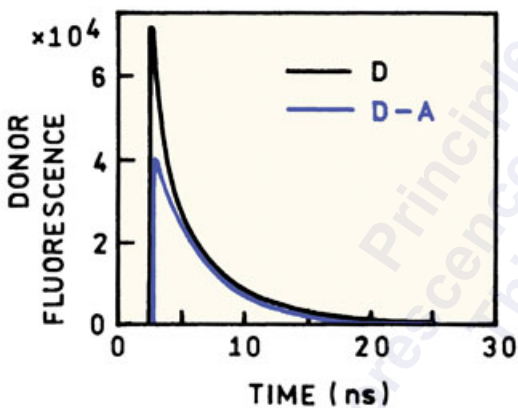


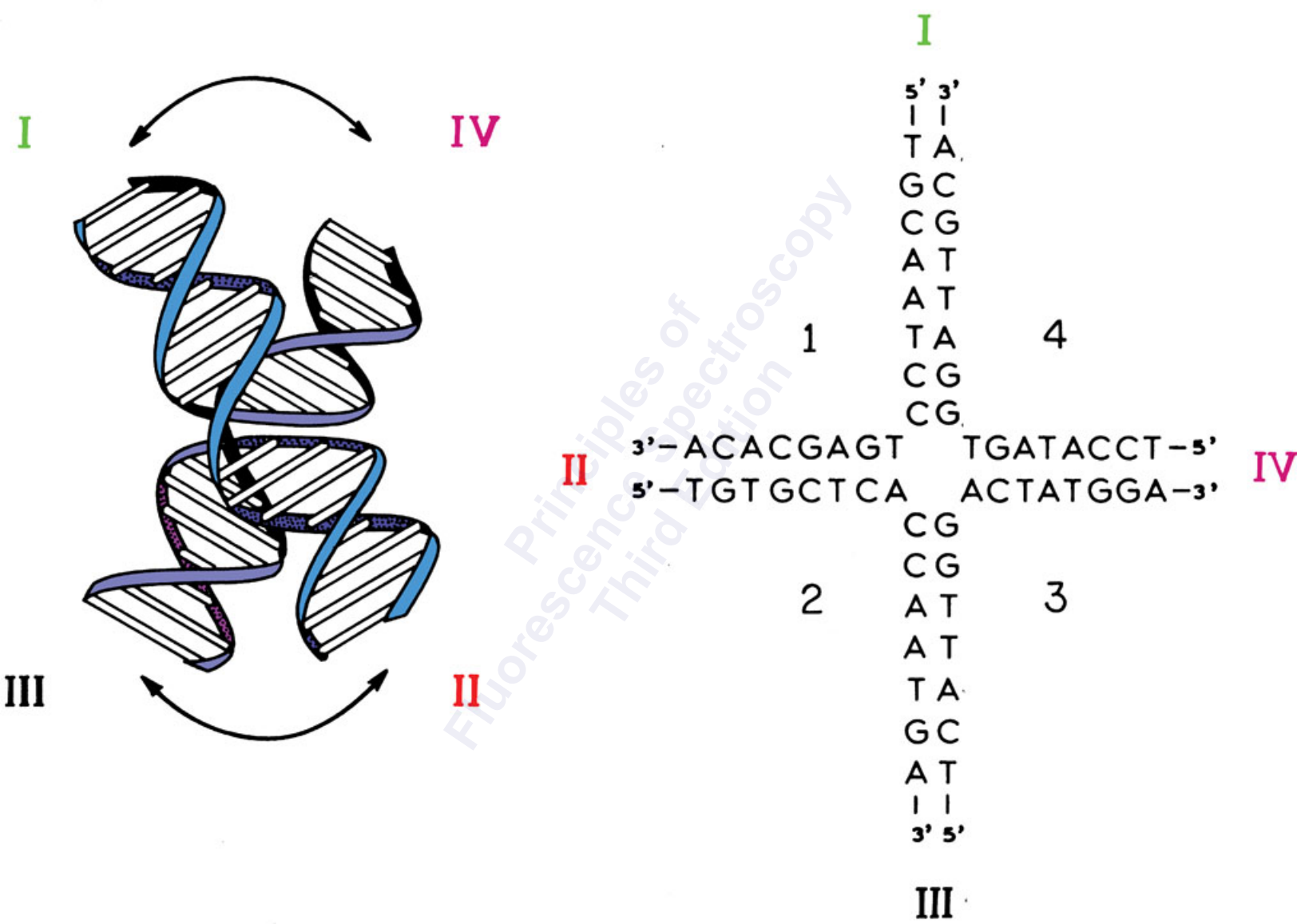




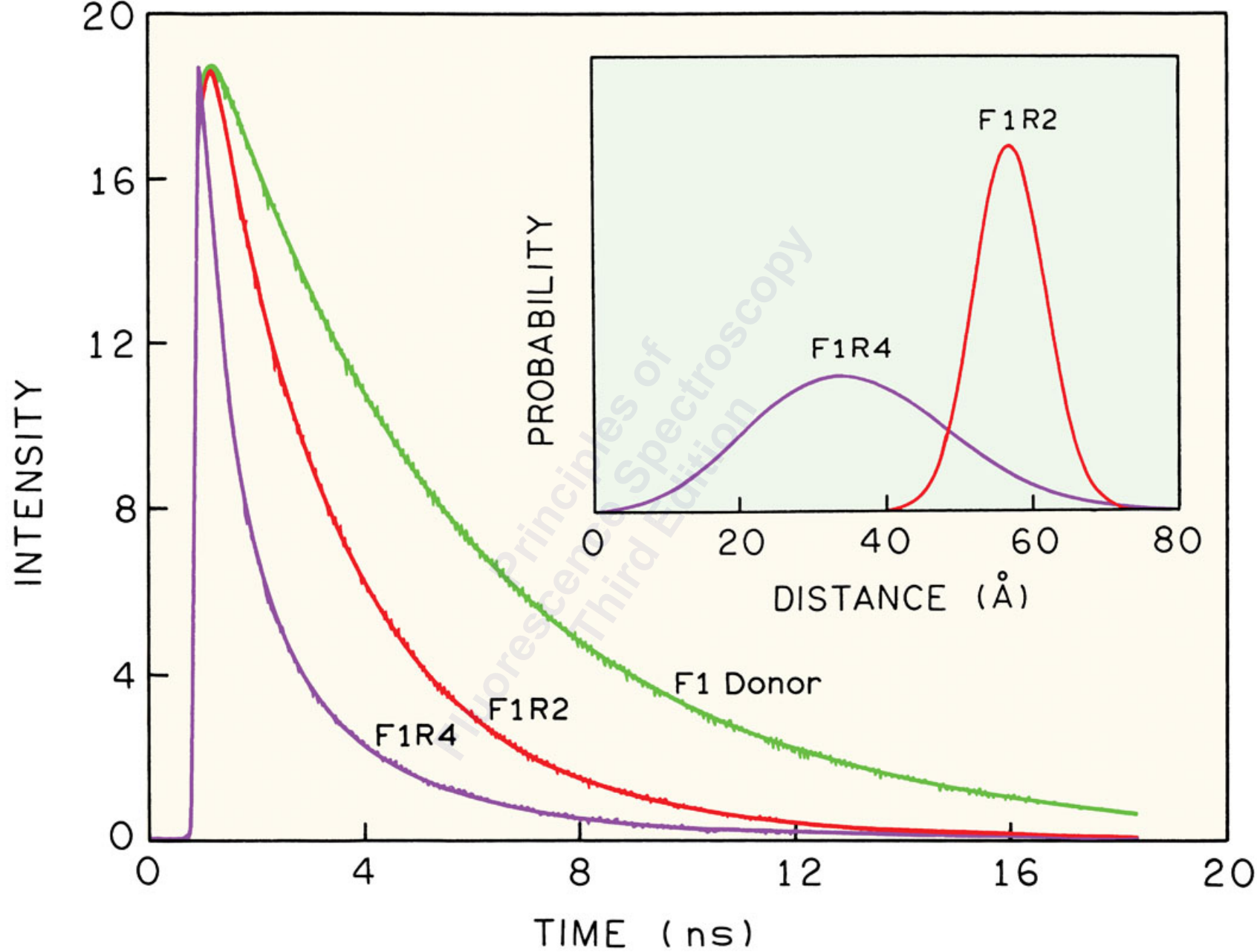
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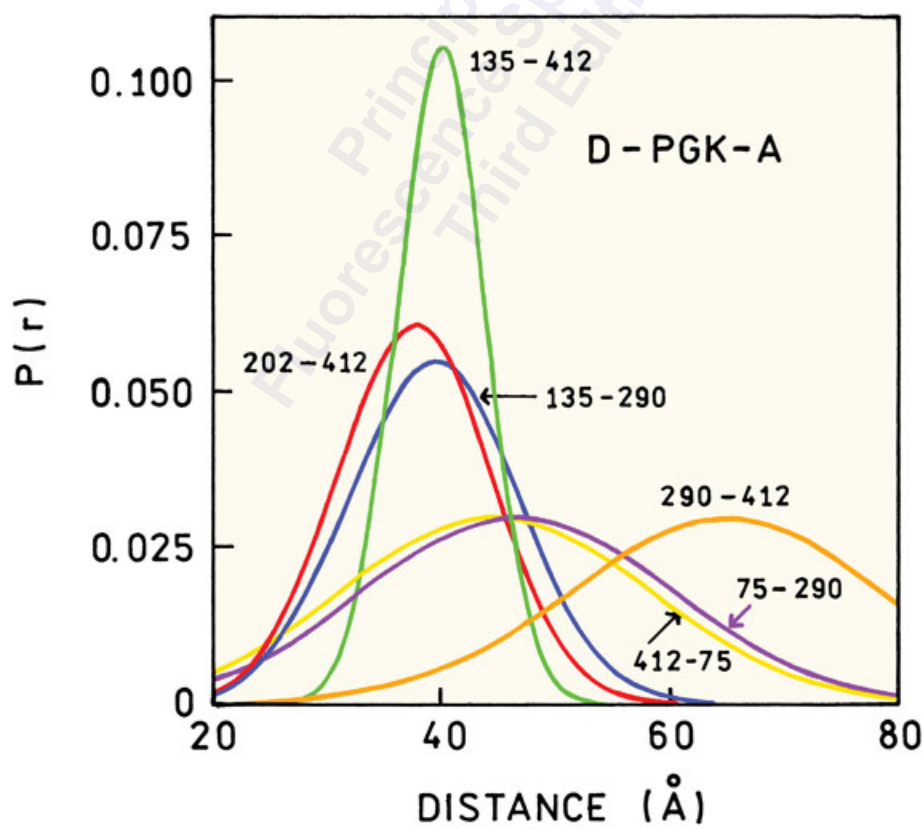
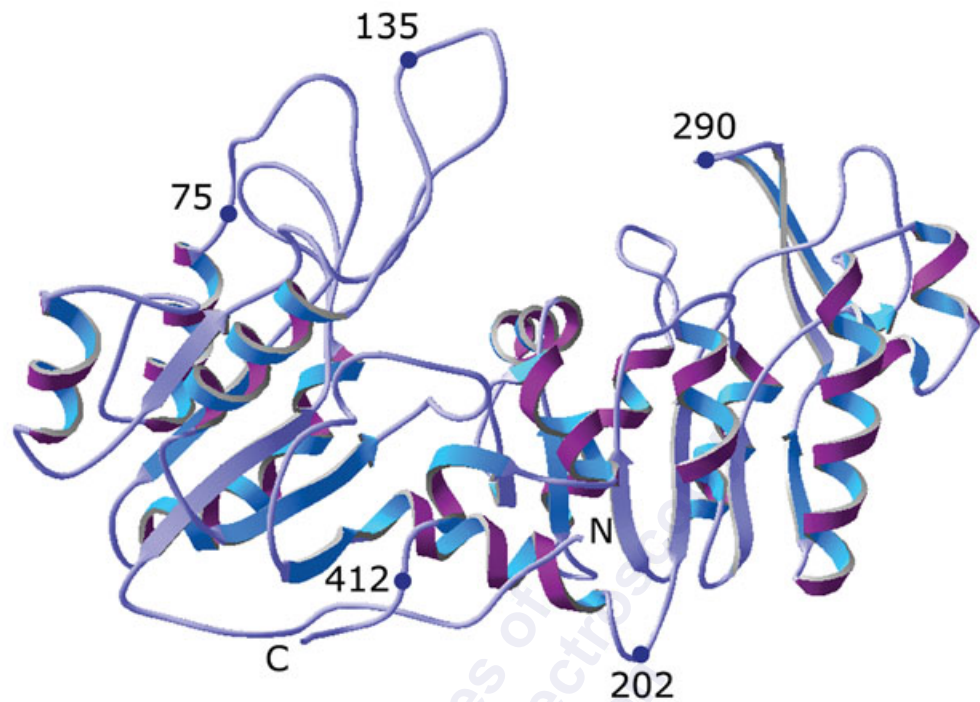
Docked

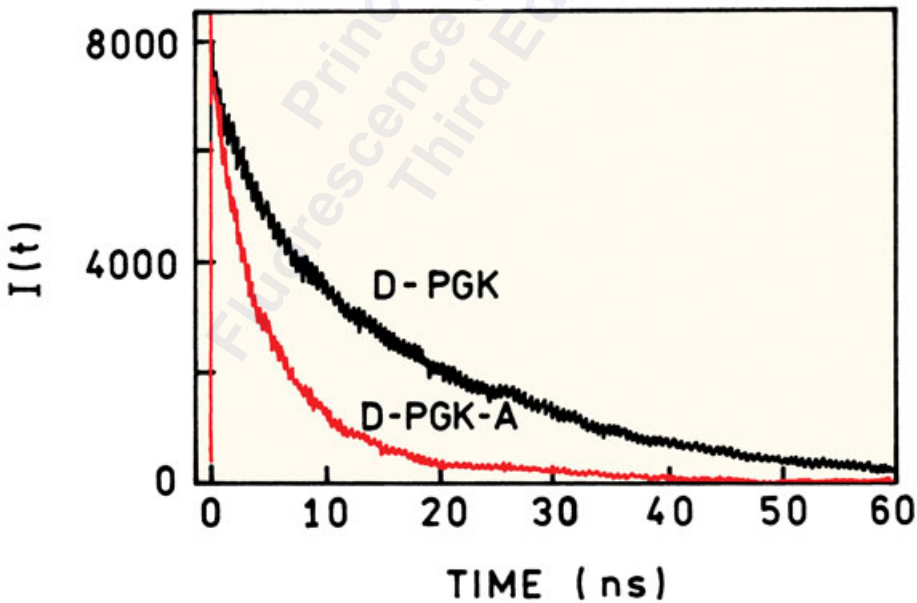
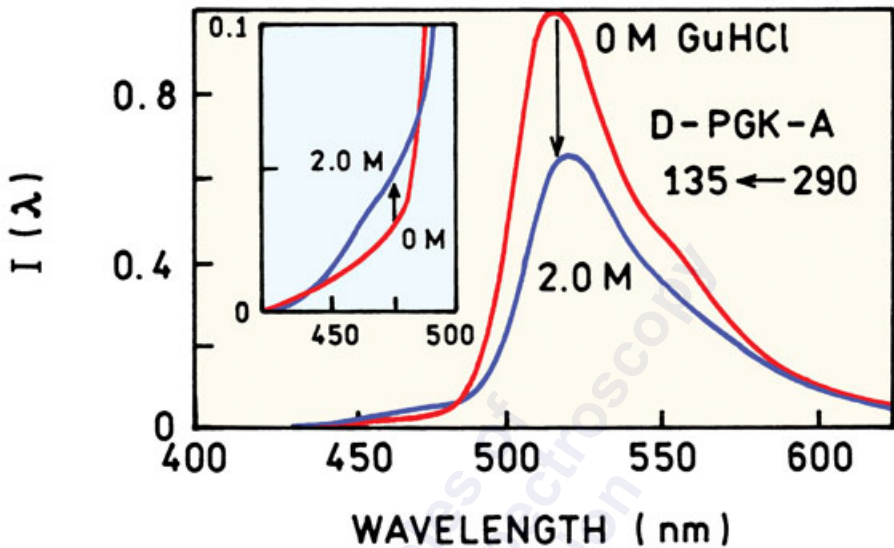


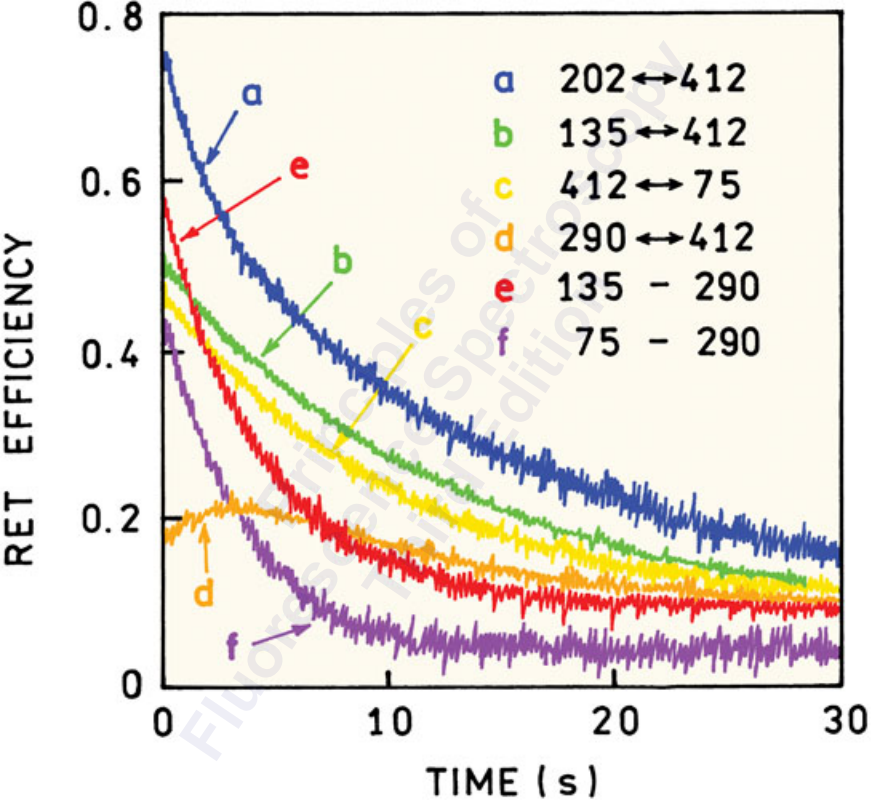






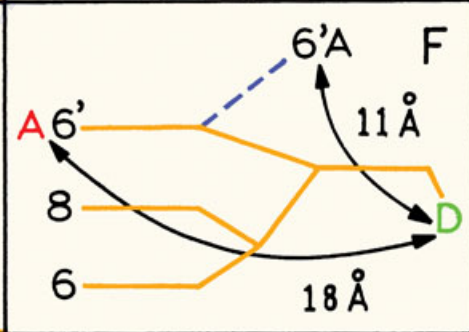
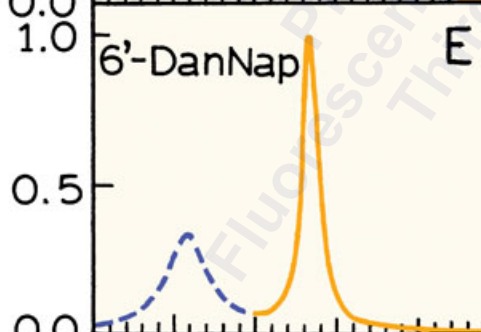
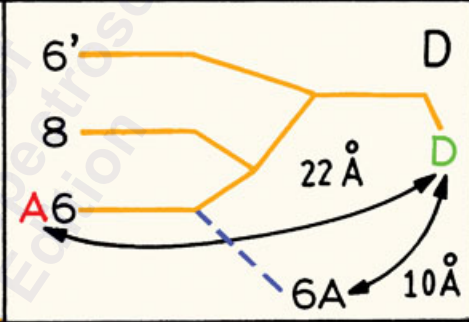
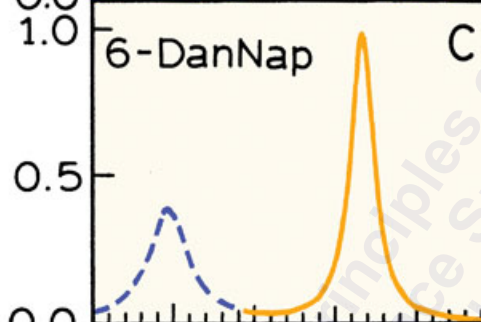
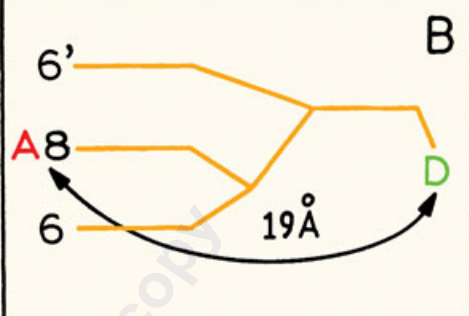
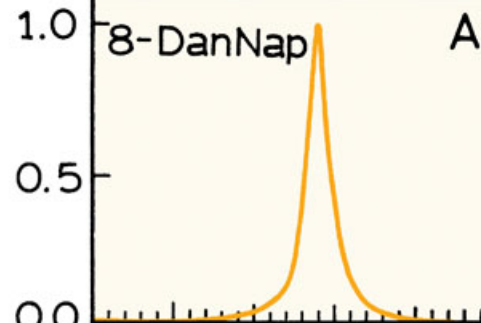






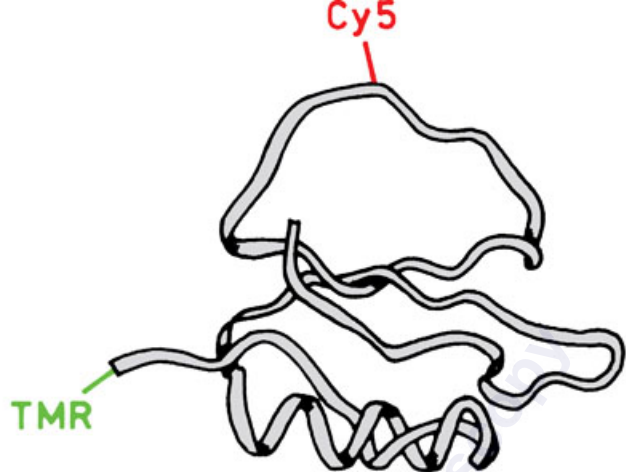


RELATIVE PROBABILITY

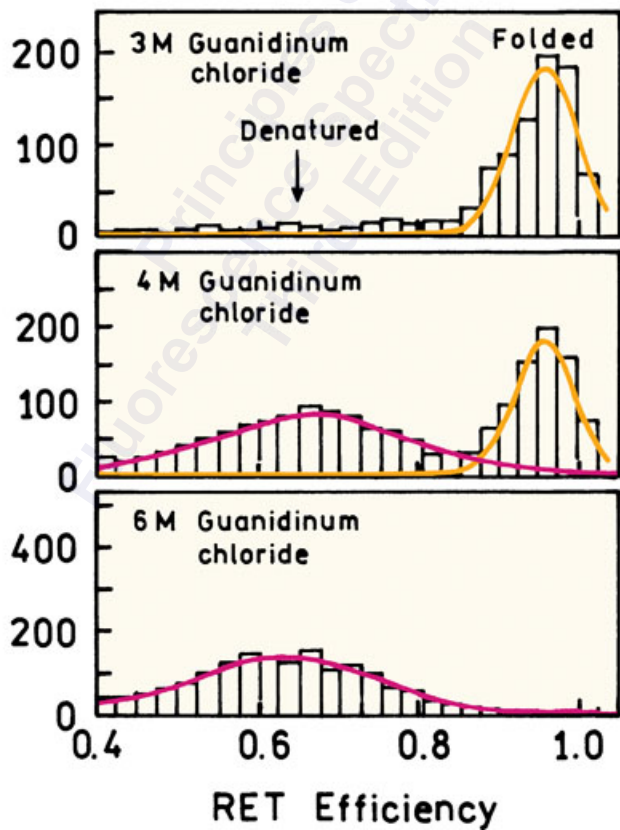


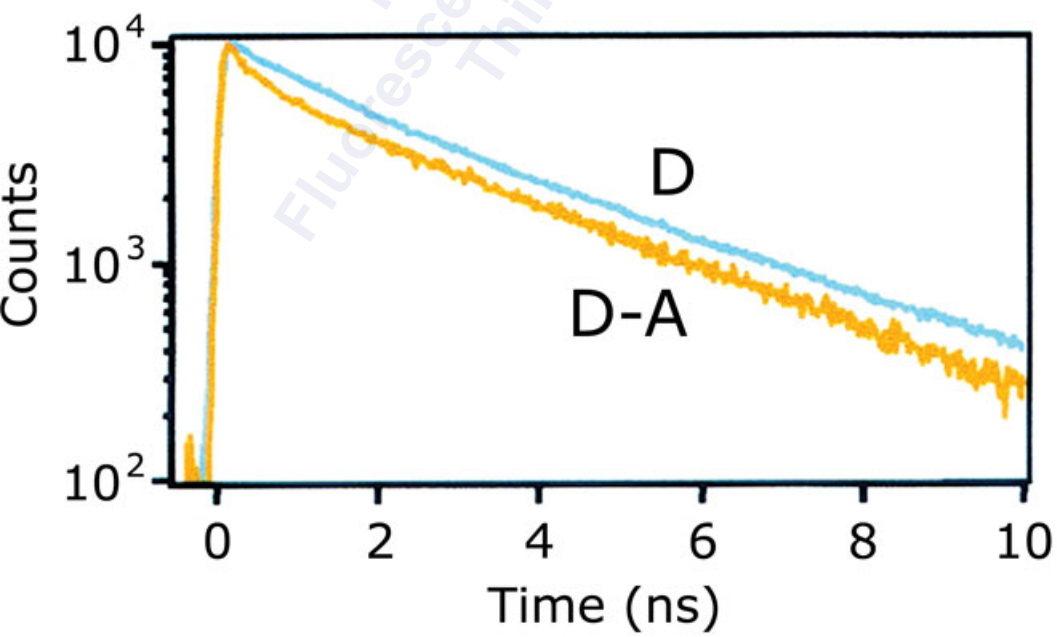
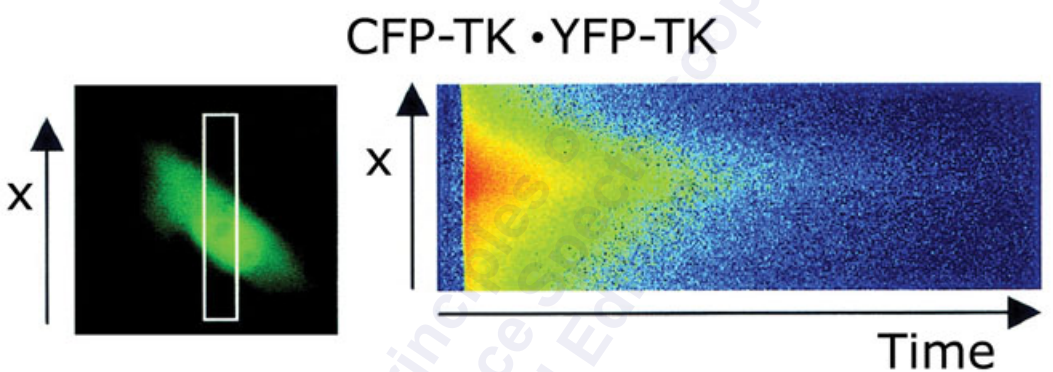
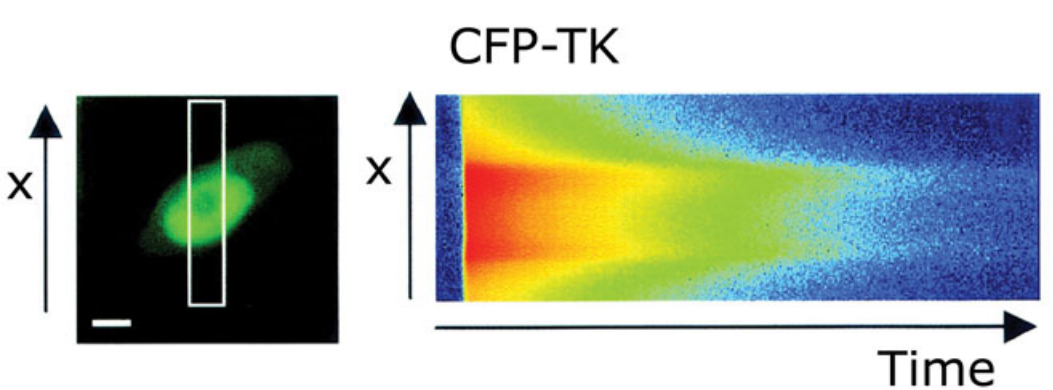
DONOR ACCEPTOR  
DISTANCE (Å)



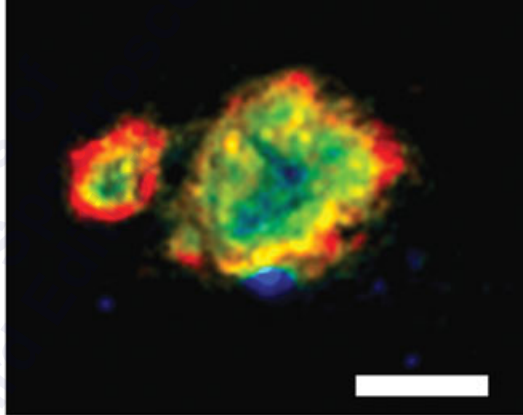
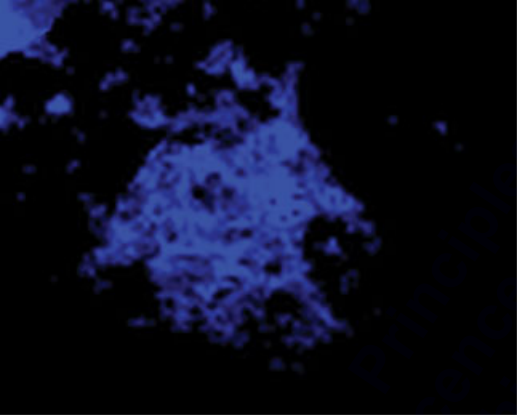


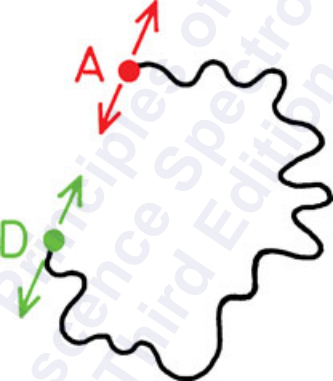
Number of Events

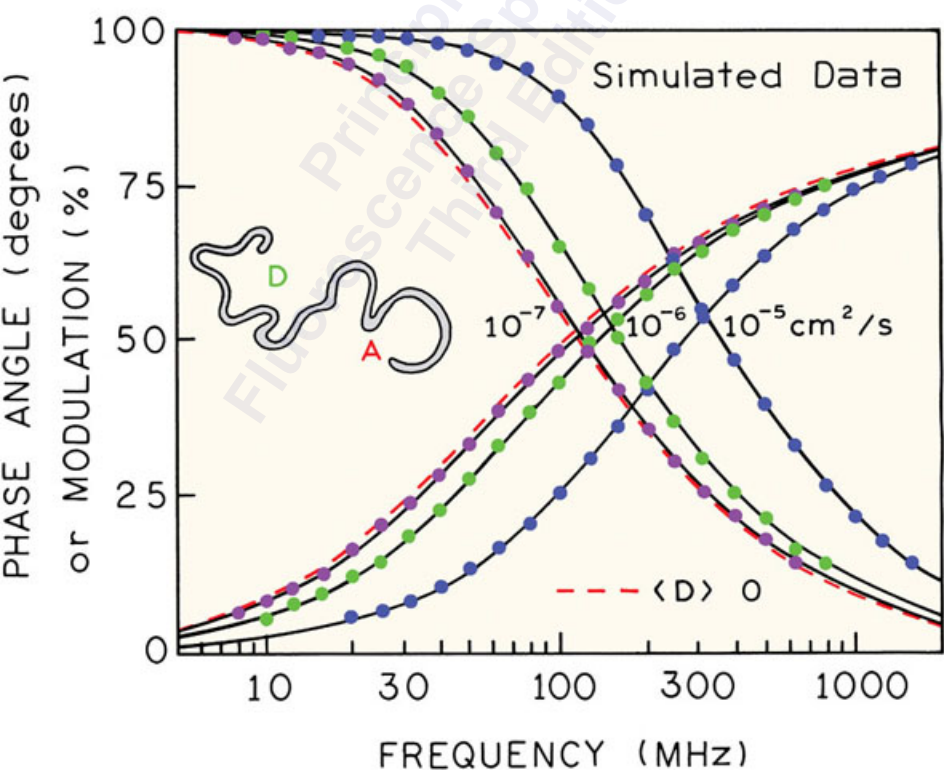
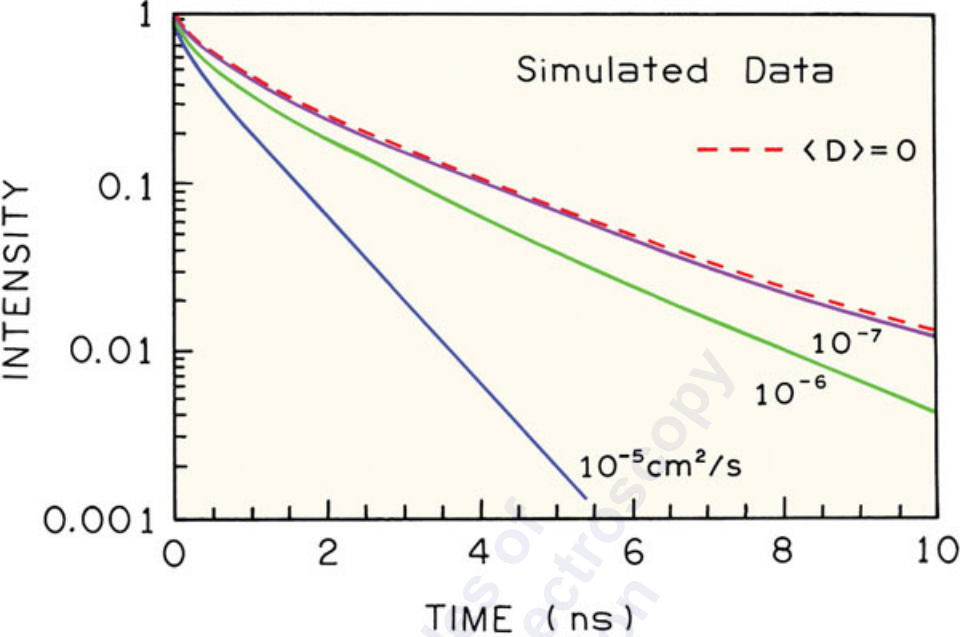


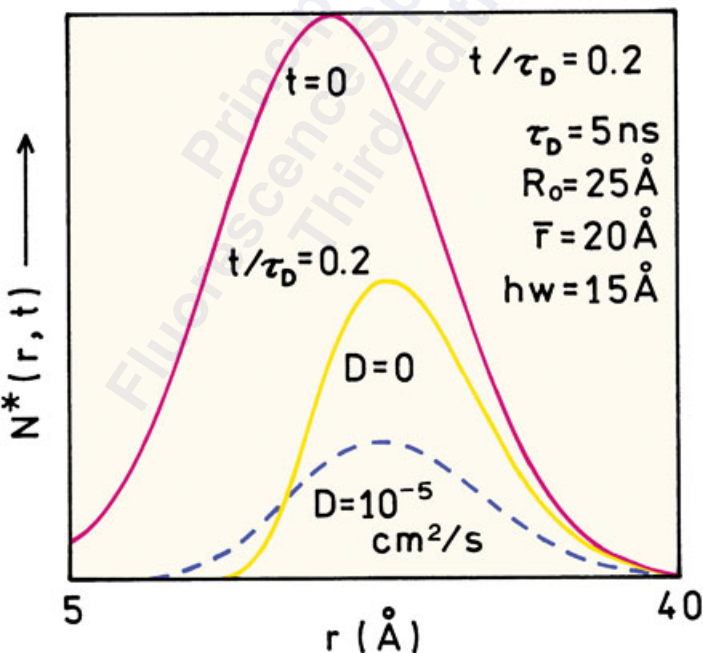
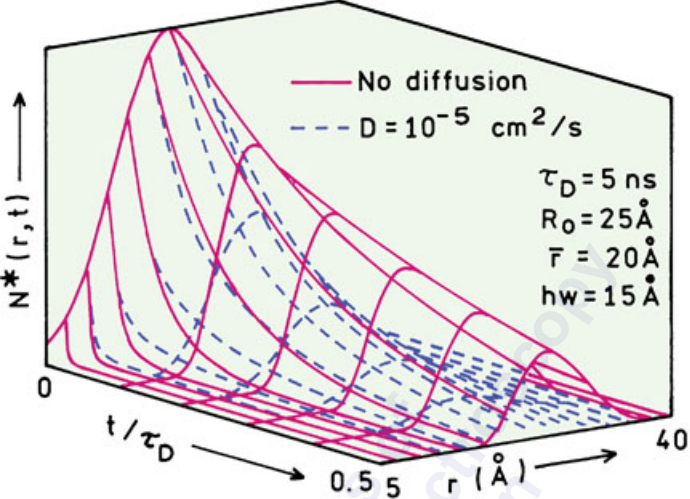


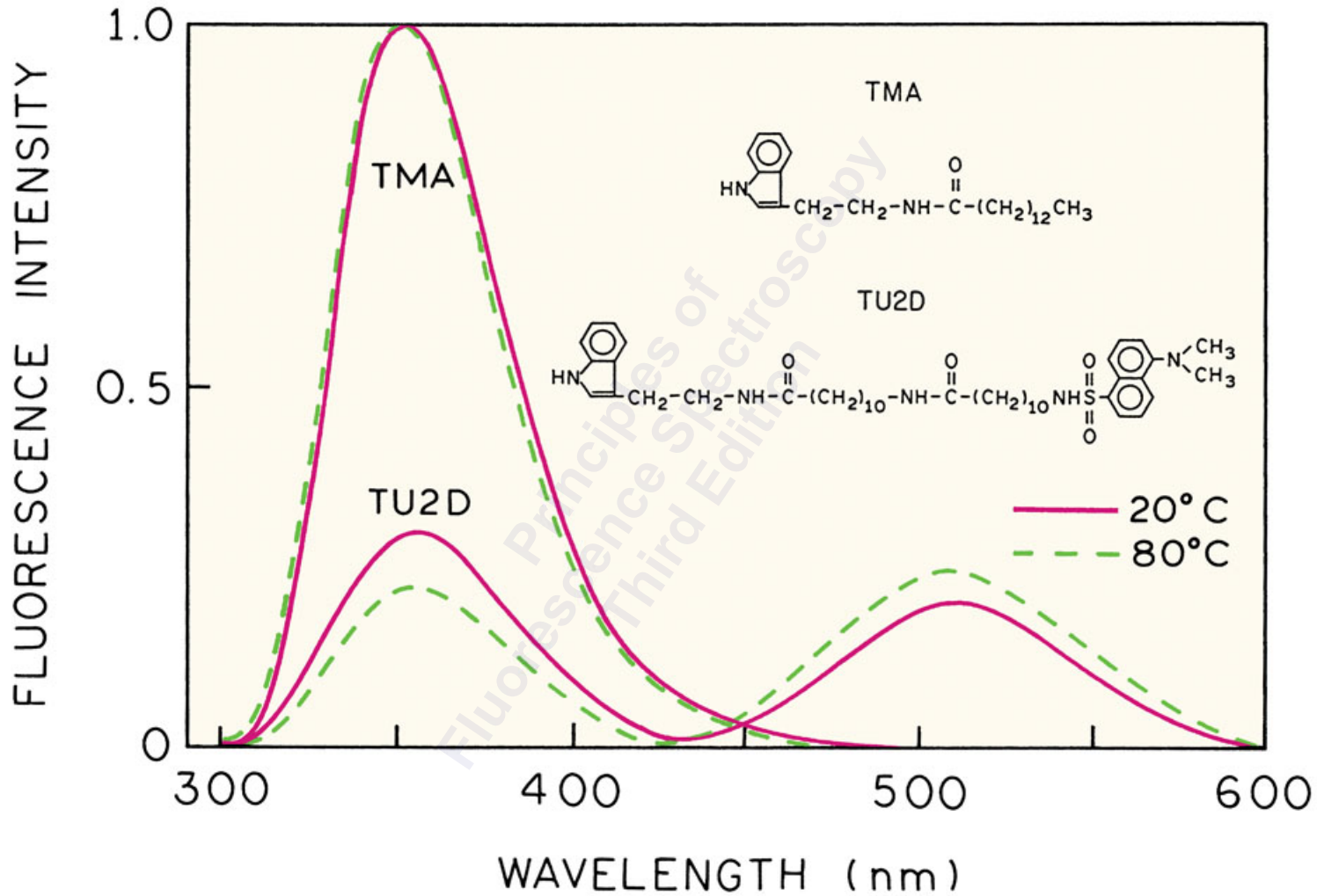


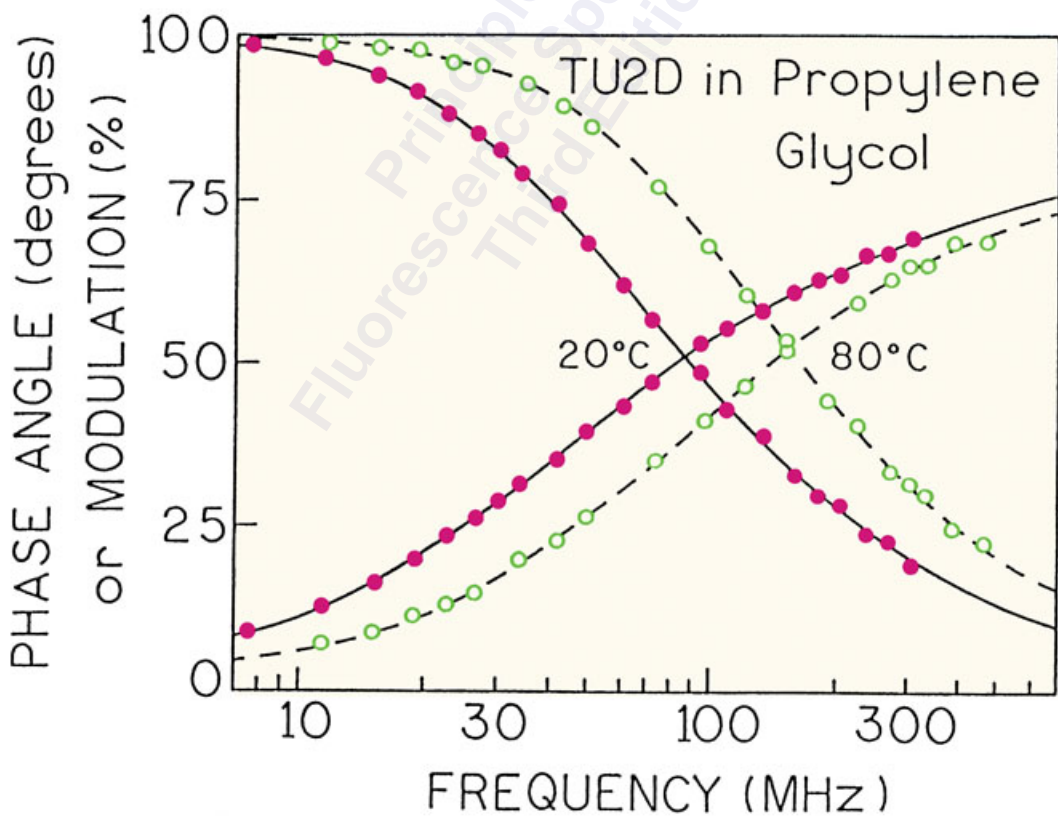
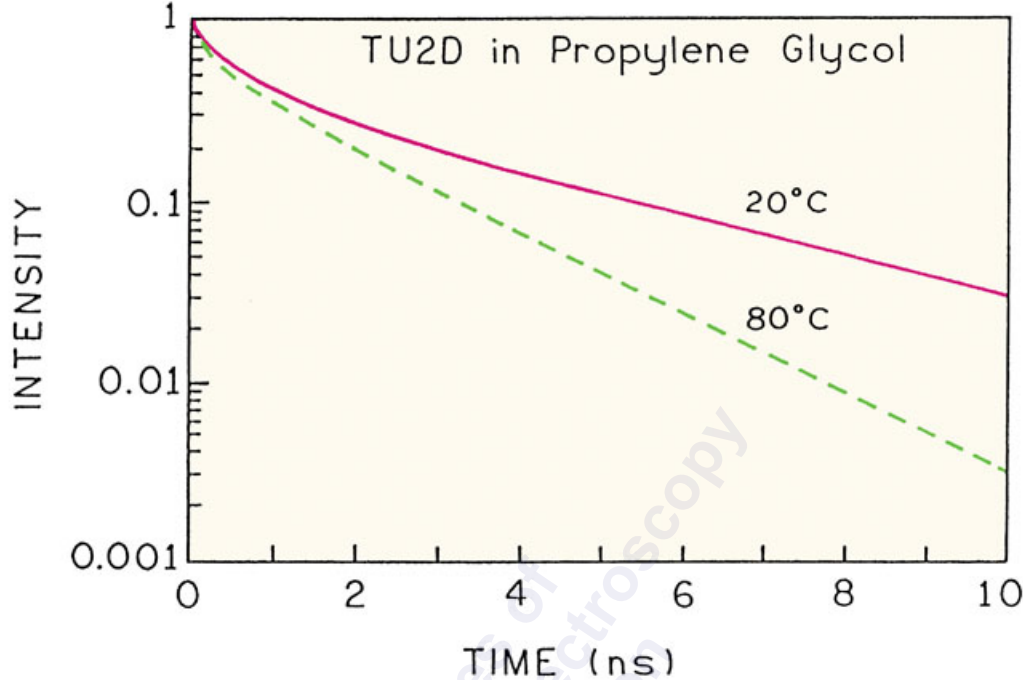


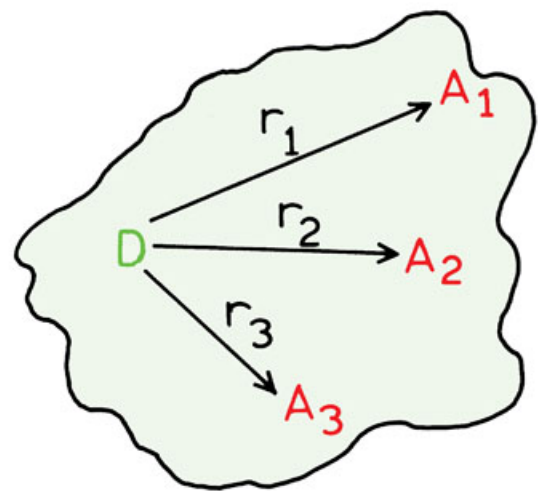




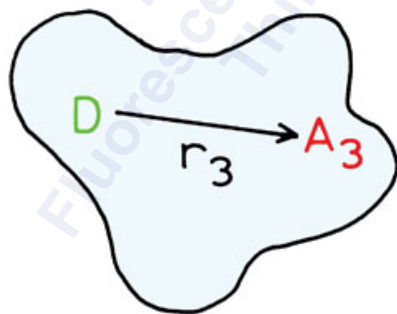
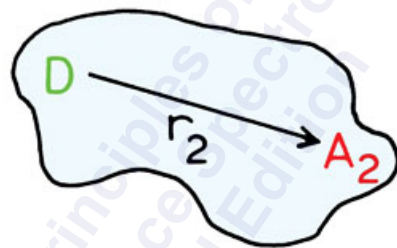
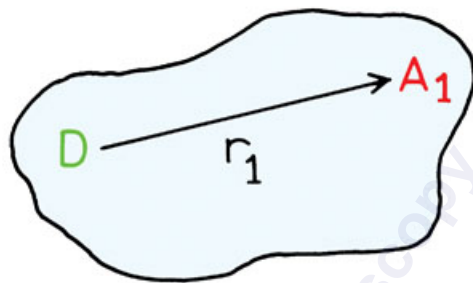




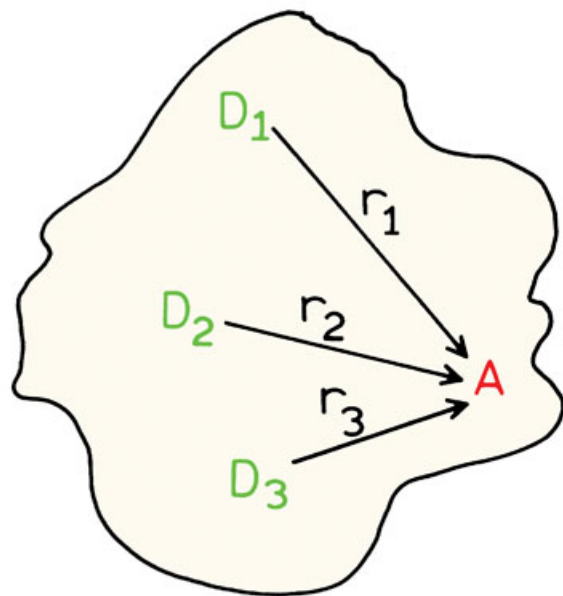




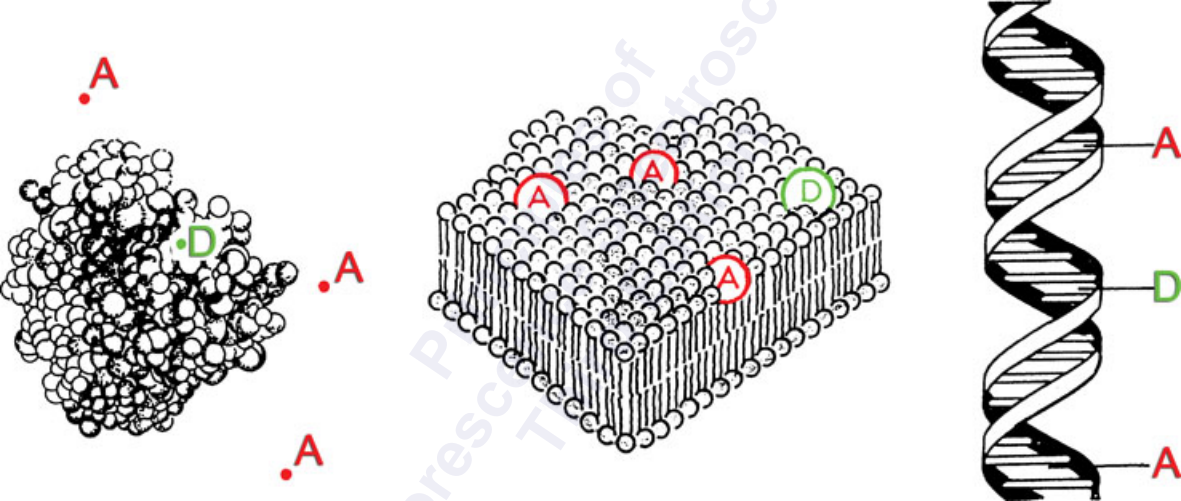
A



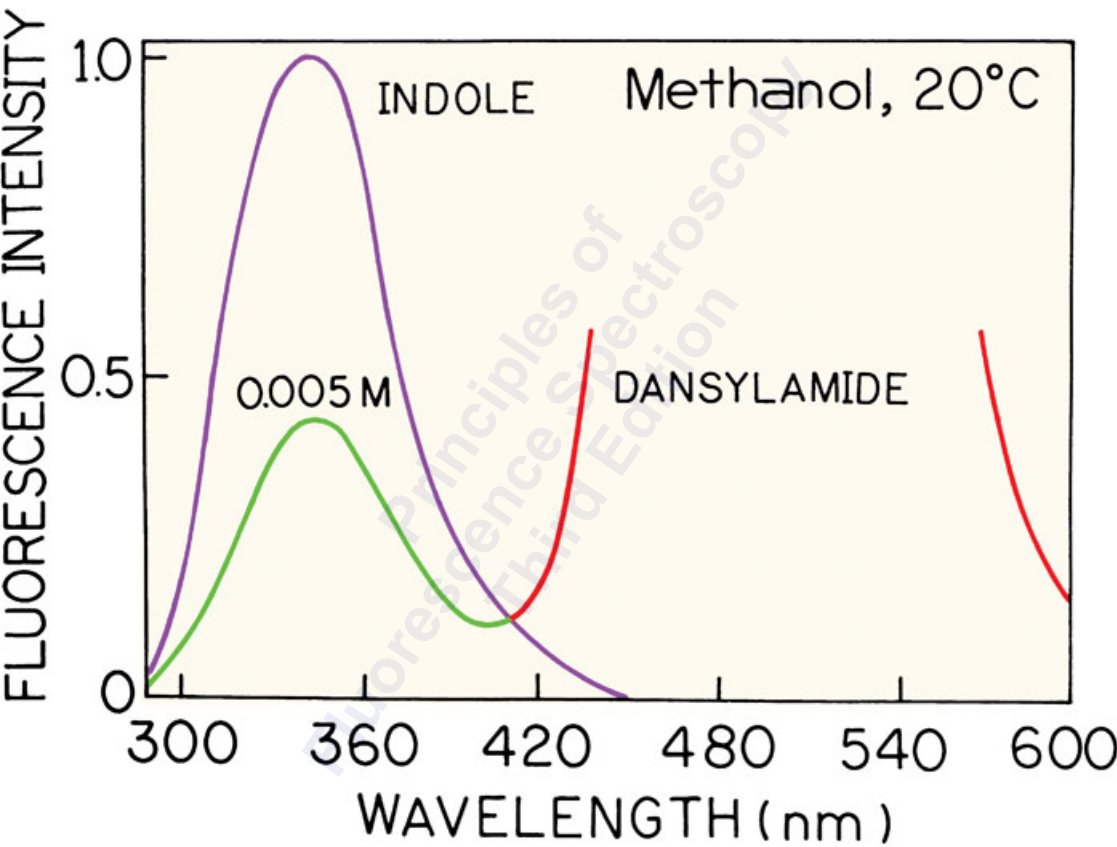
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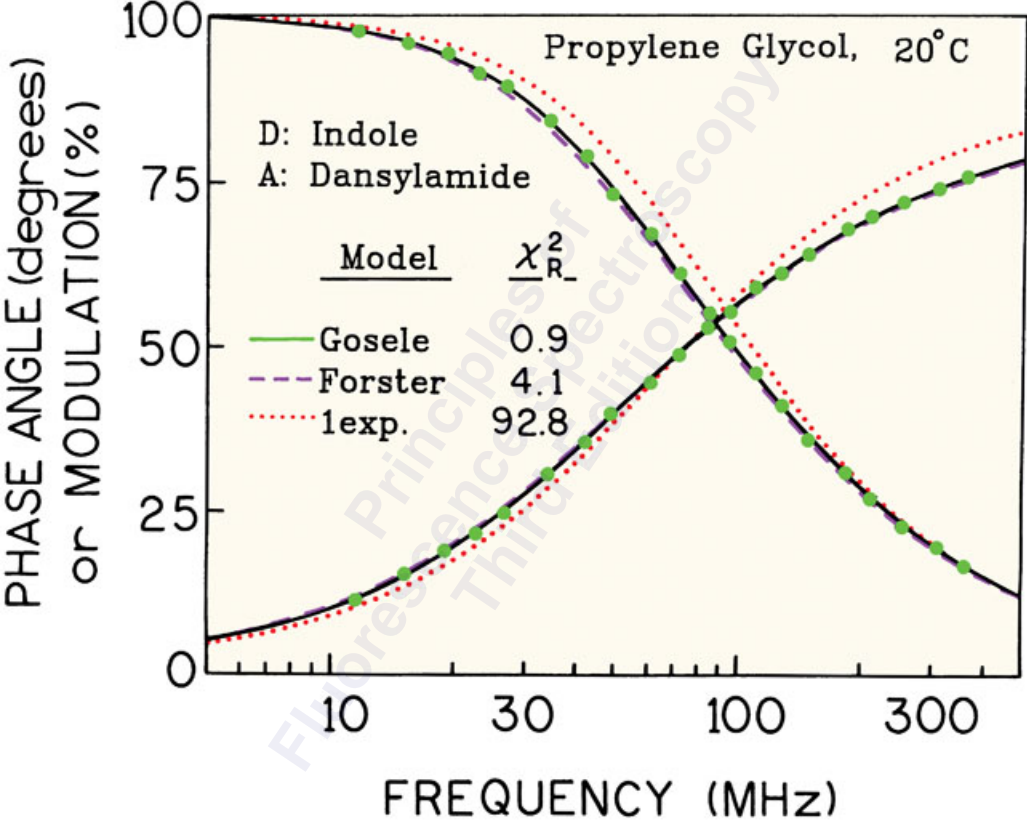


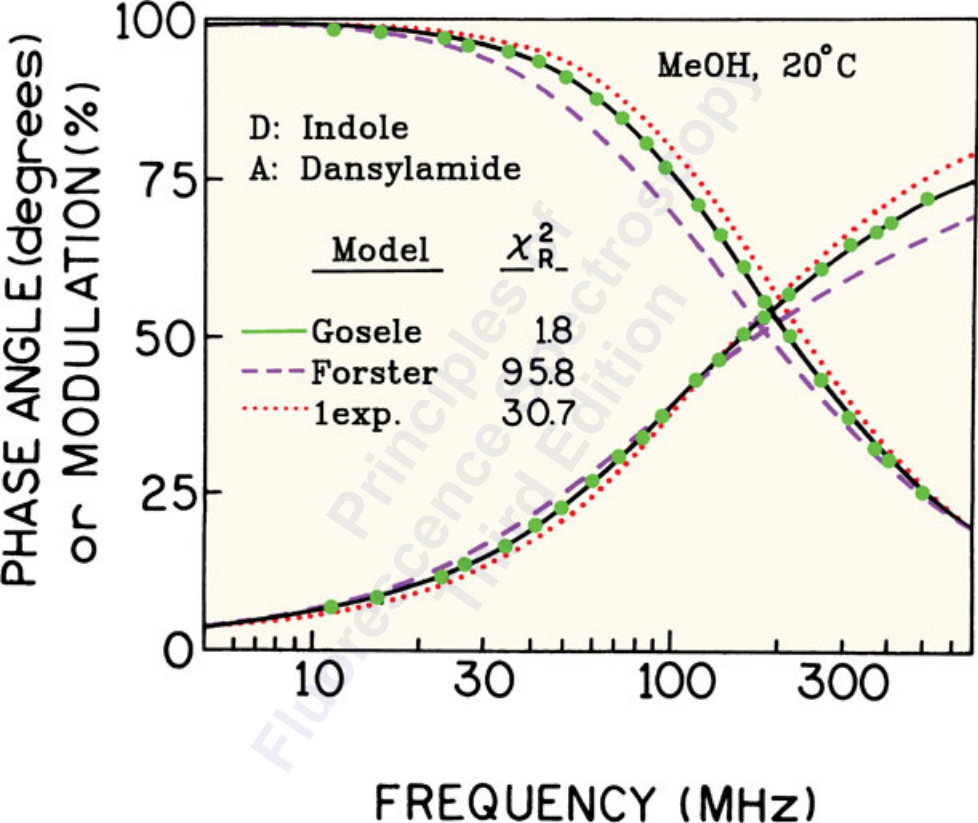
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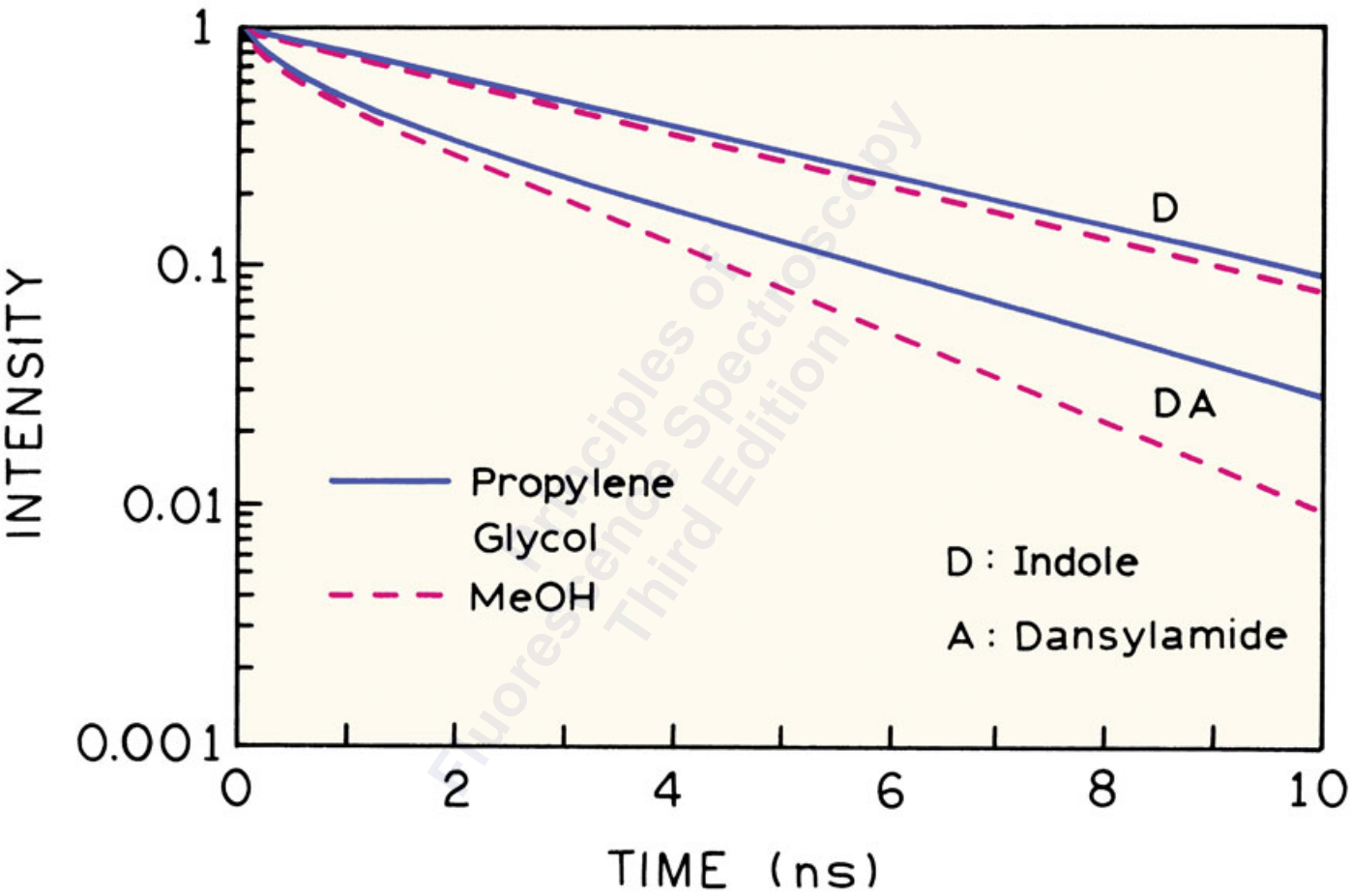


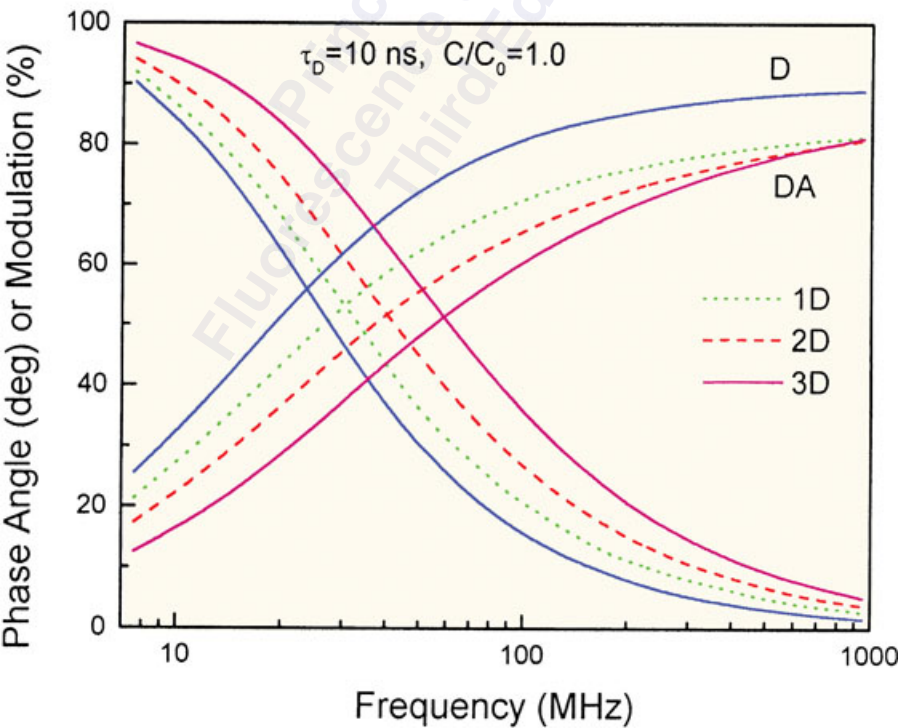
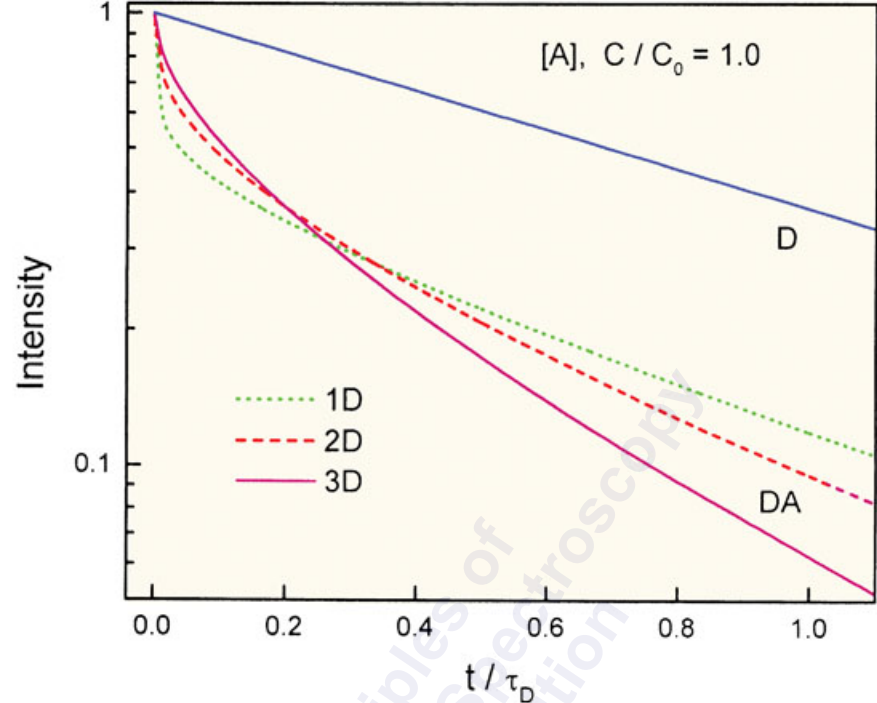


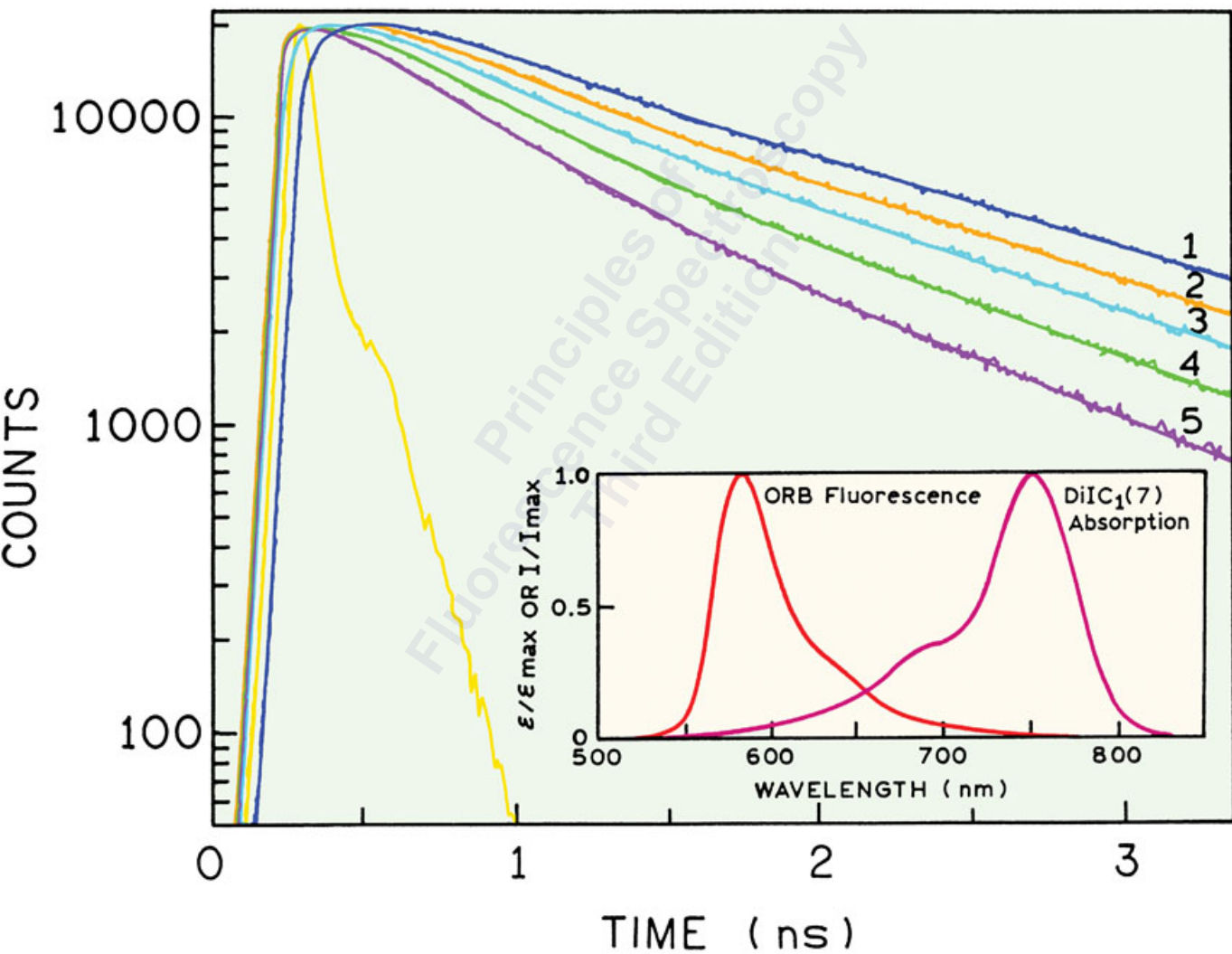
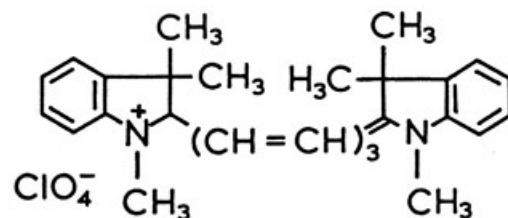
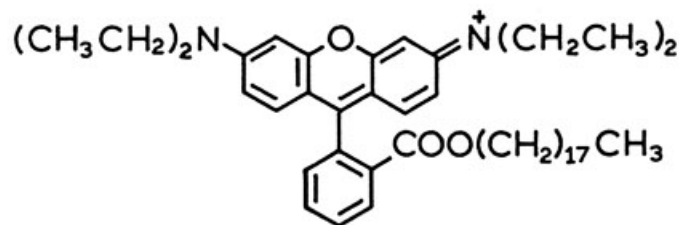


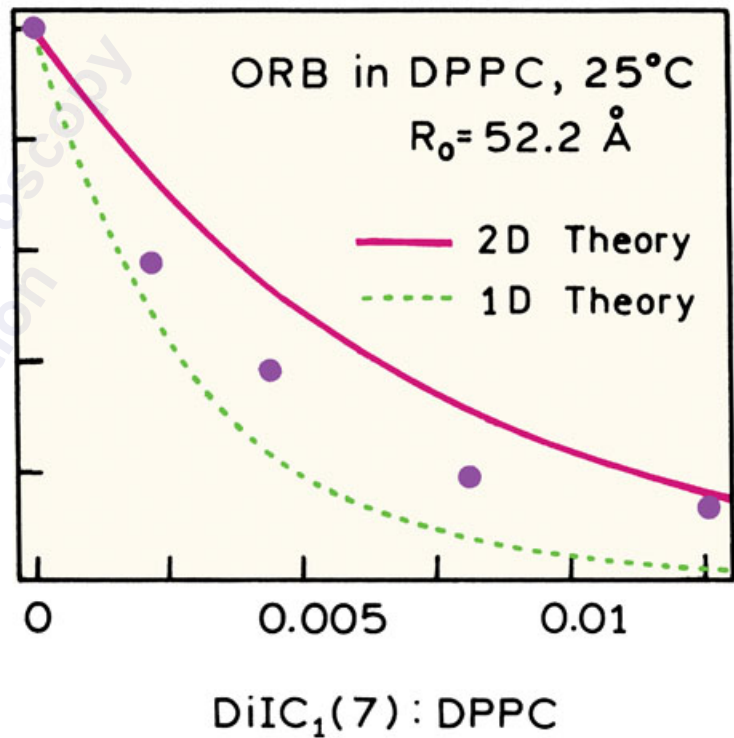
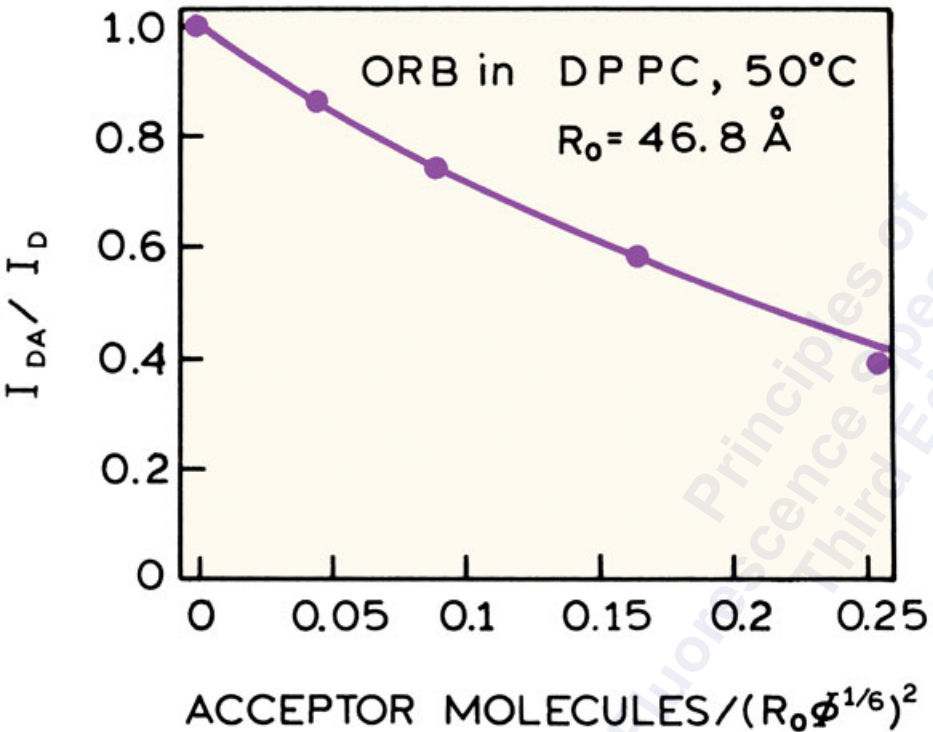




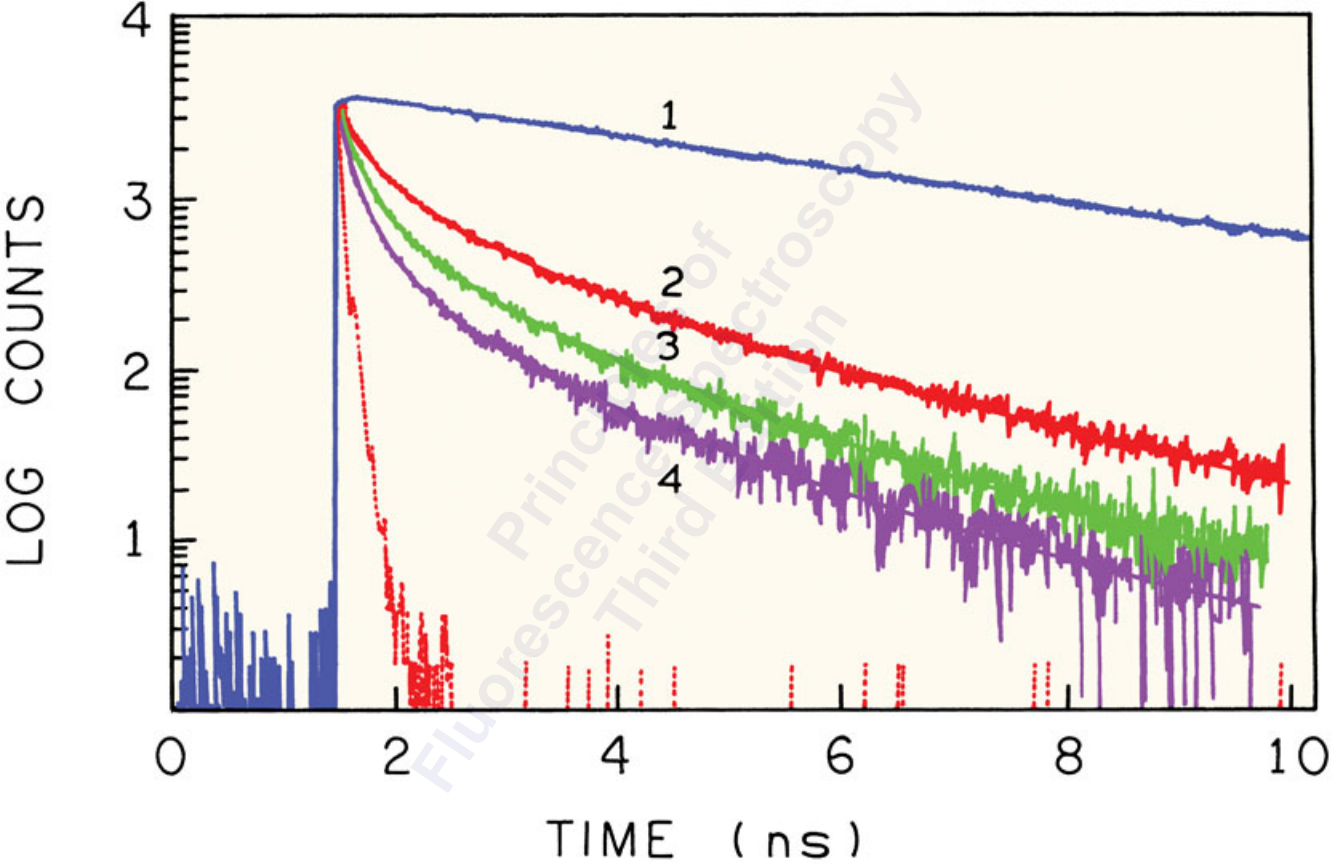






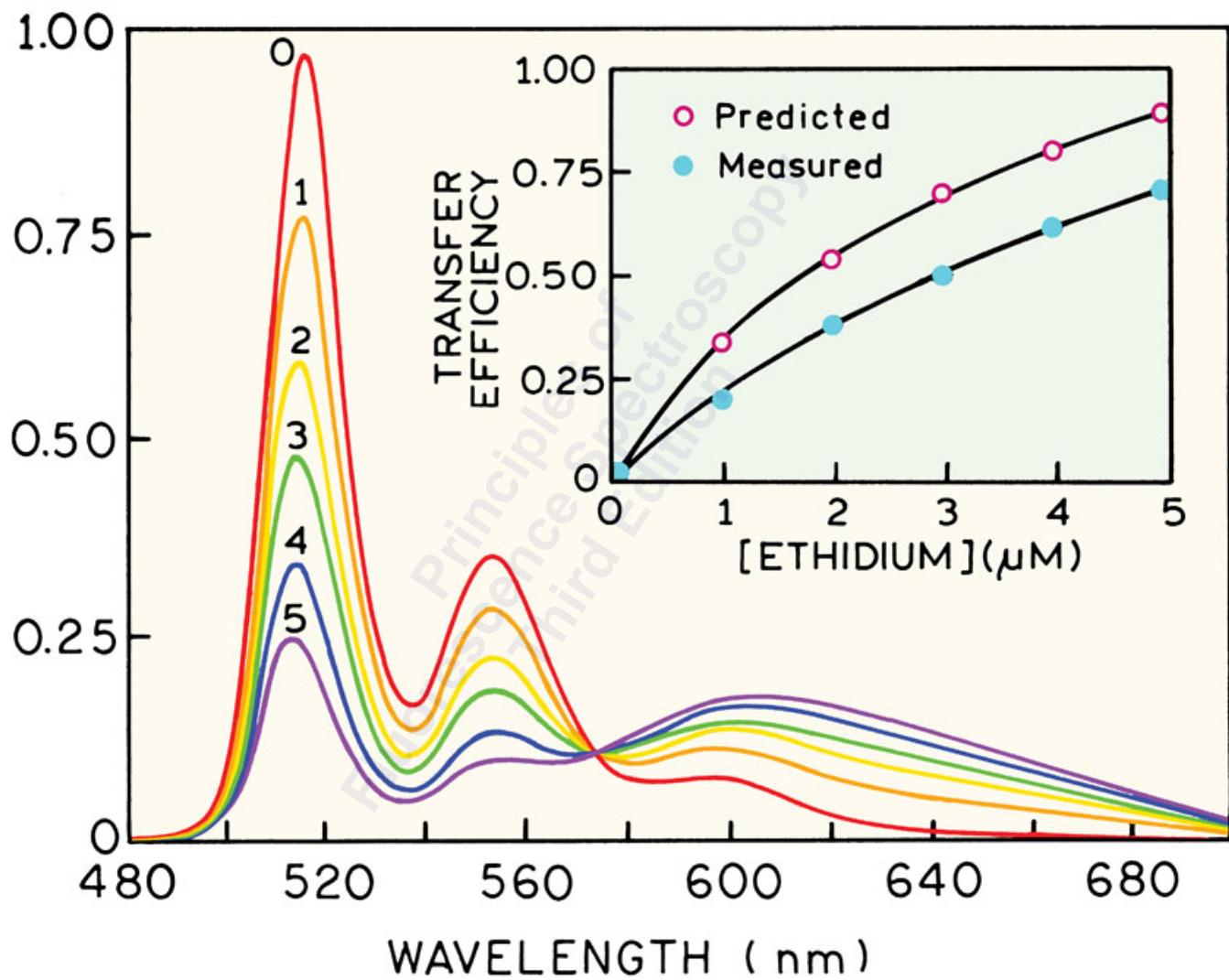


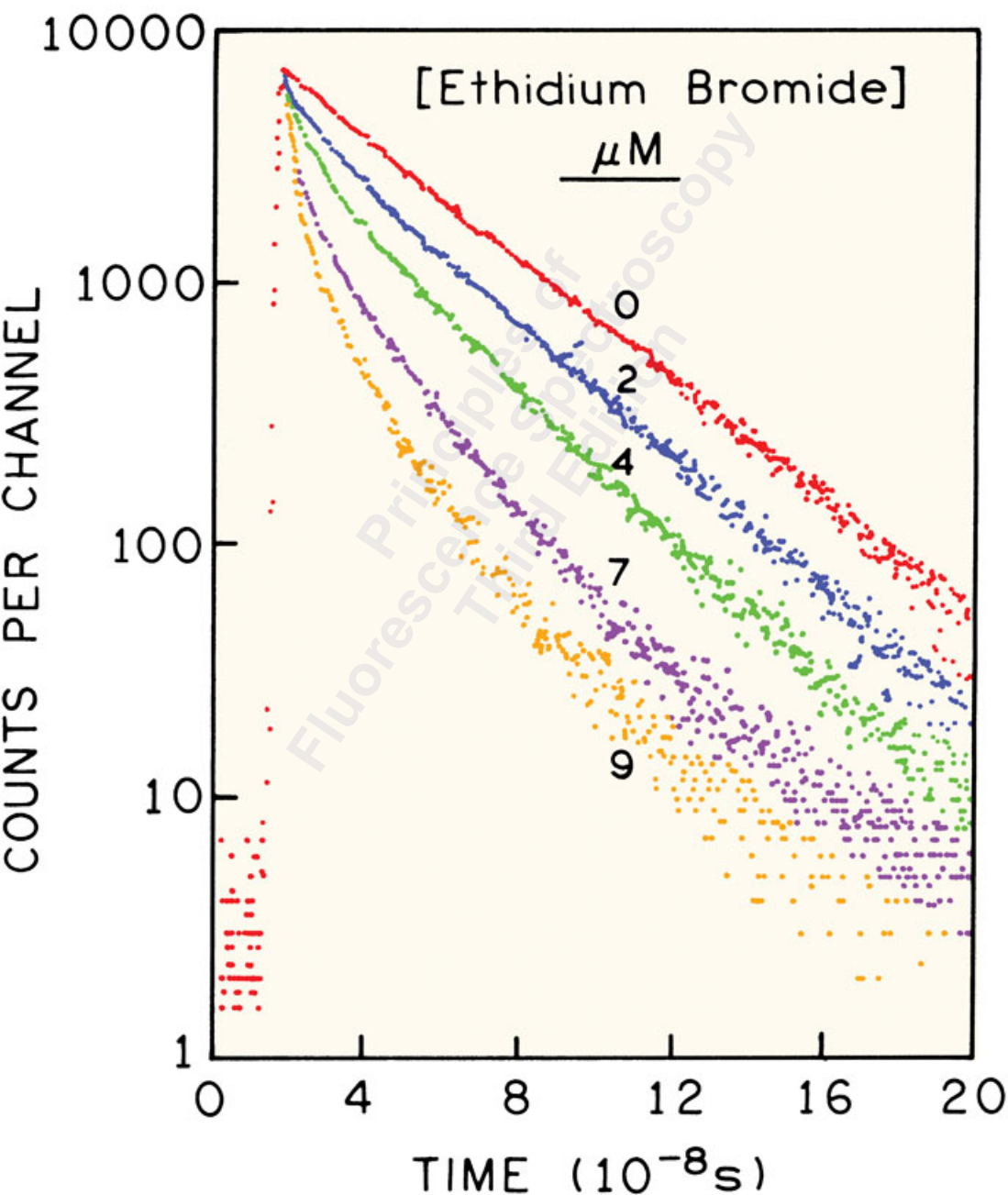
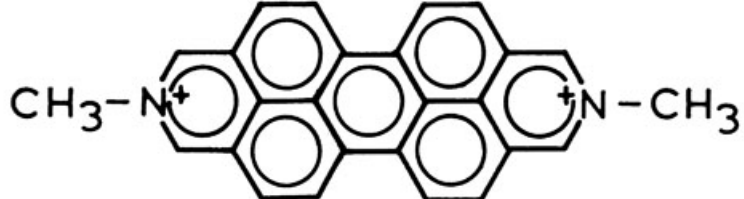






EMISSION FLUORESCENCE INTENSITY (a.u.)





FLUORESCENCE

Ex : 470nm

Donor : AO

Acceptor: NB

NB 0

0.033

0.043

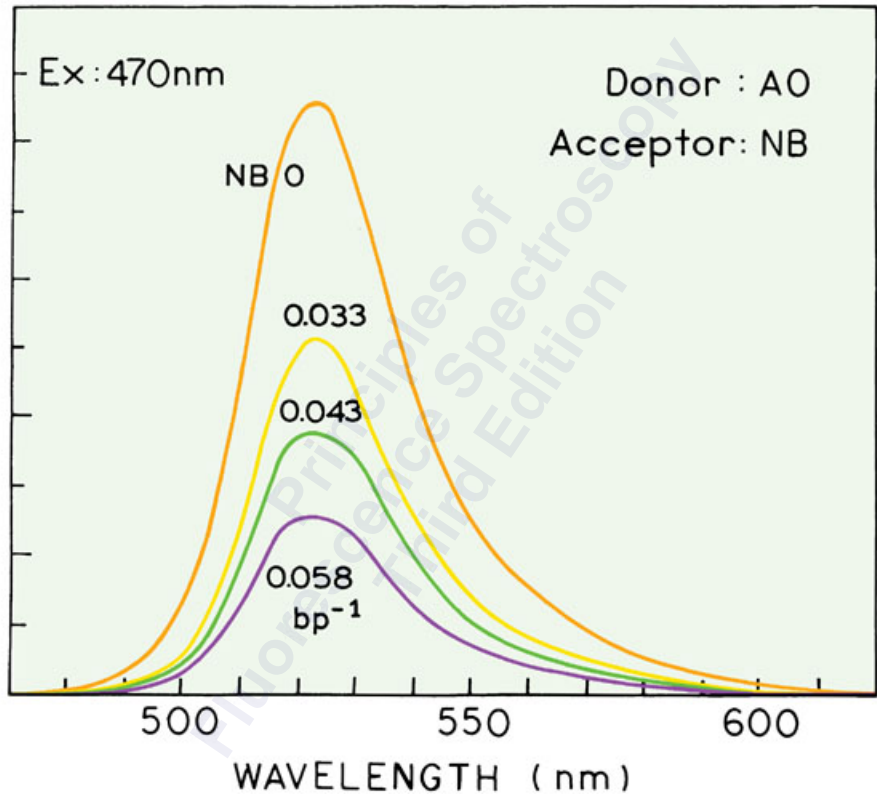
0.058  
bp<sup>-1</sup>

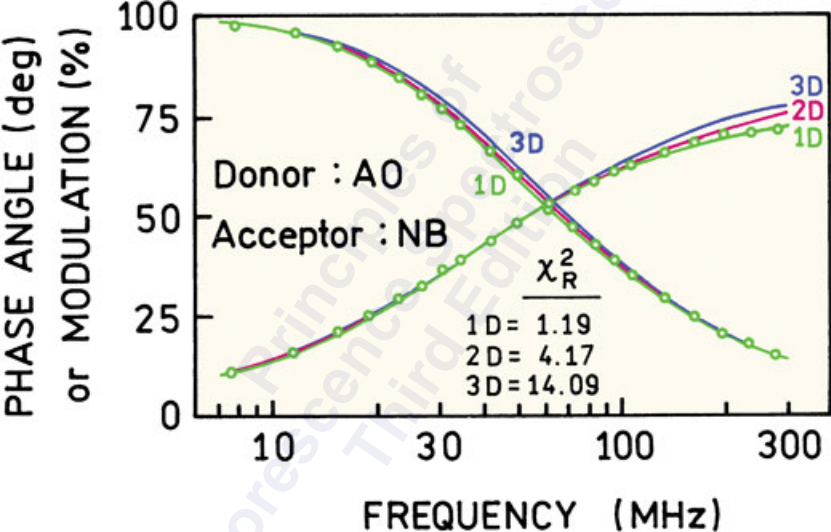
500

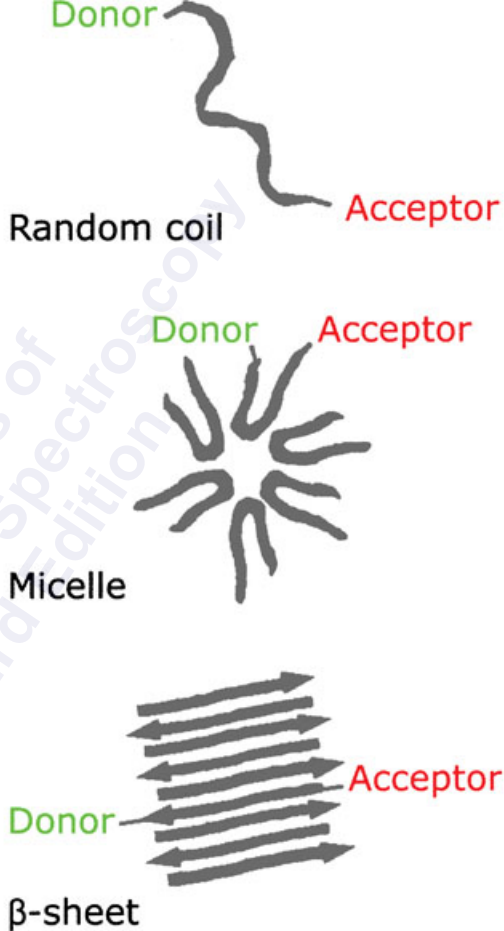
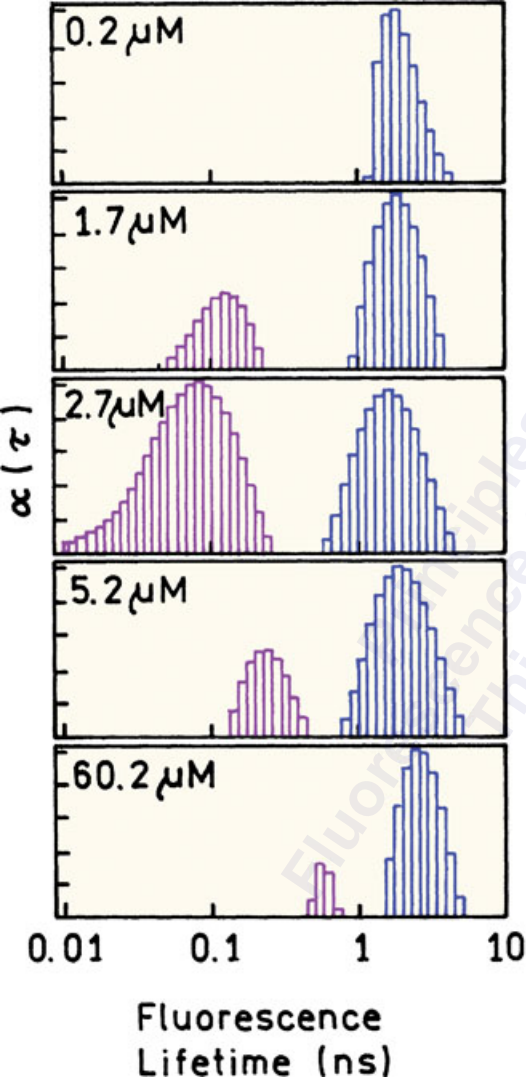
550

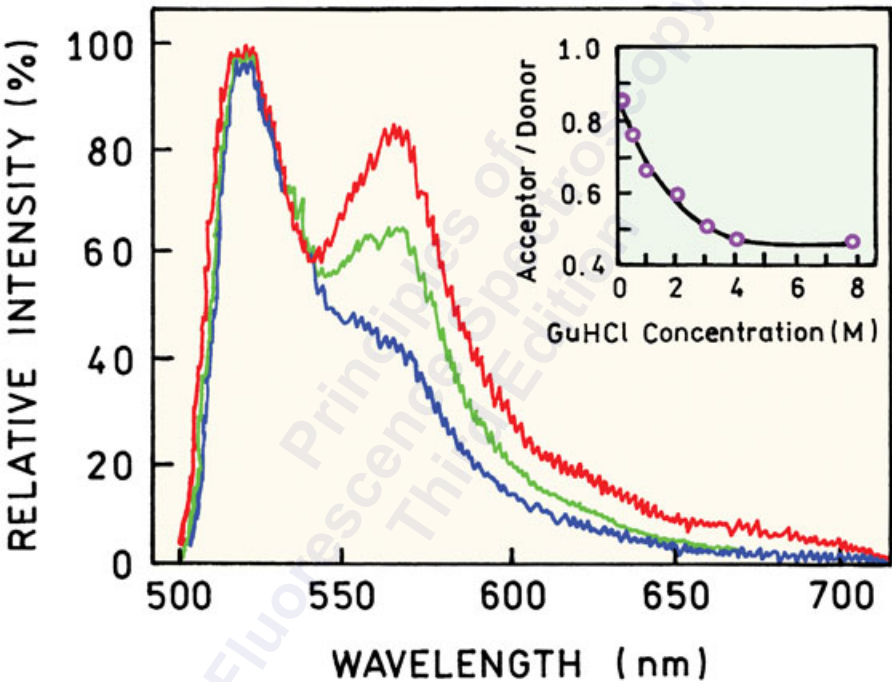
600

WAVELENGTH (nm)

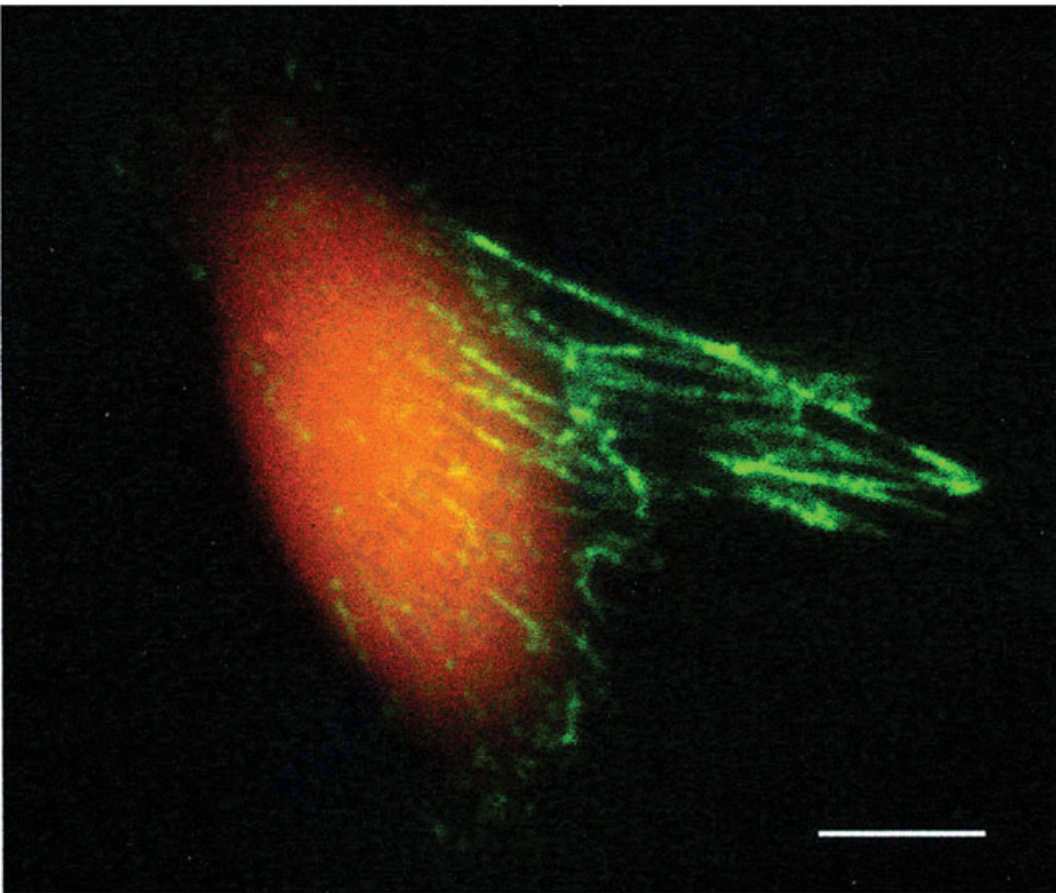


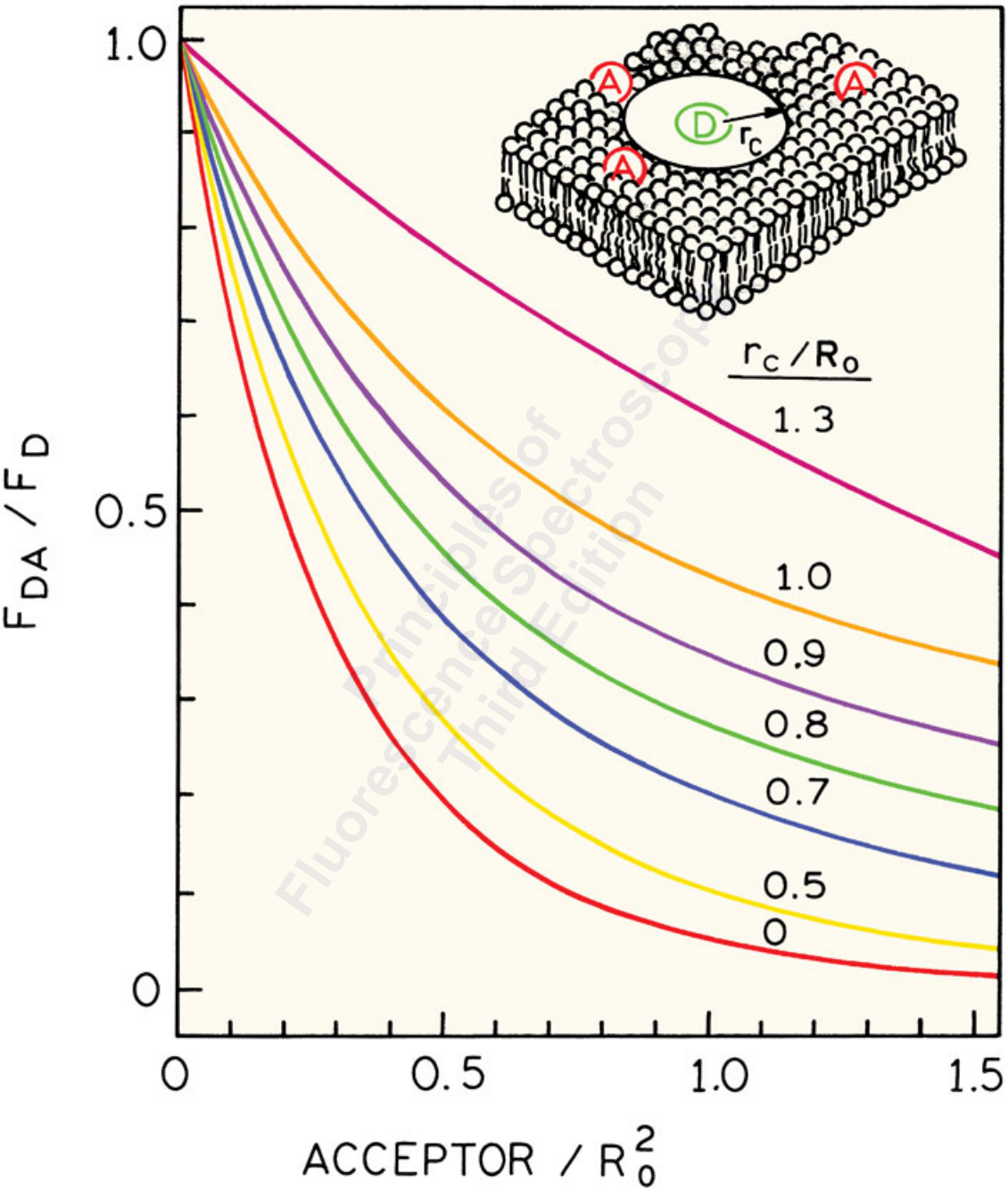




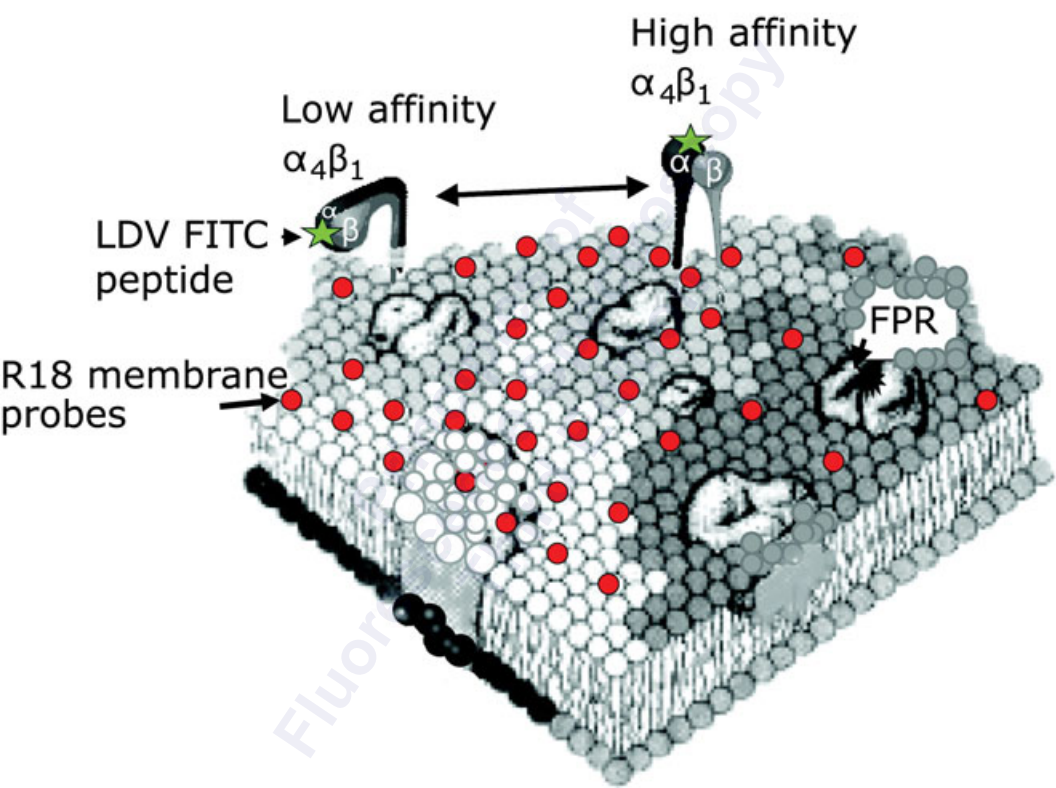


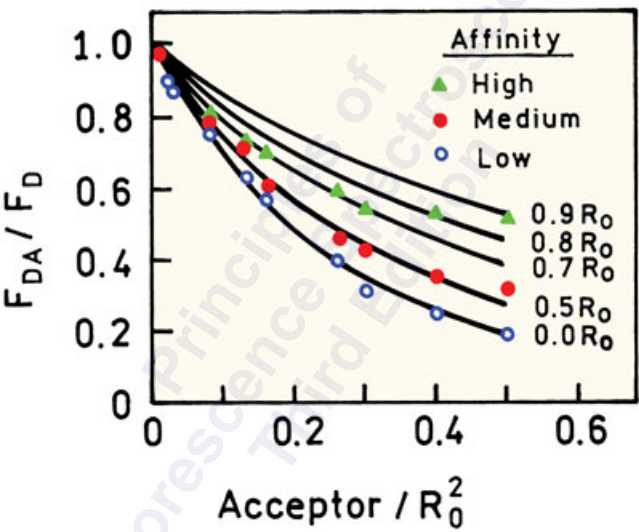


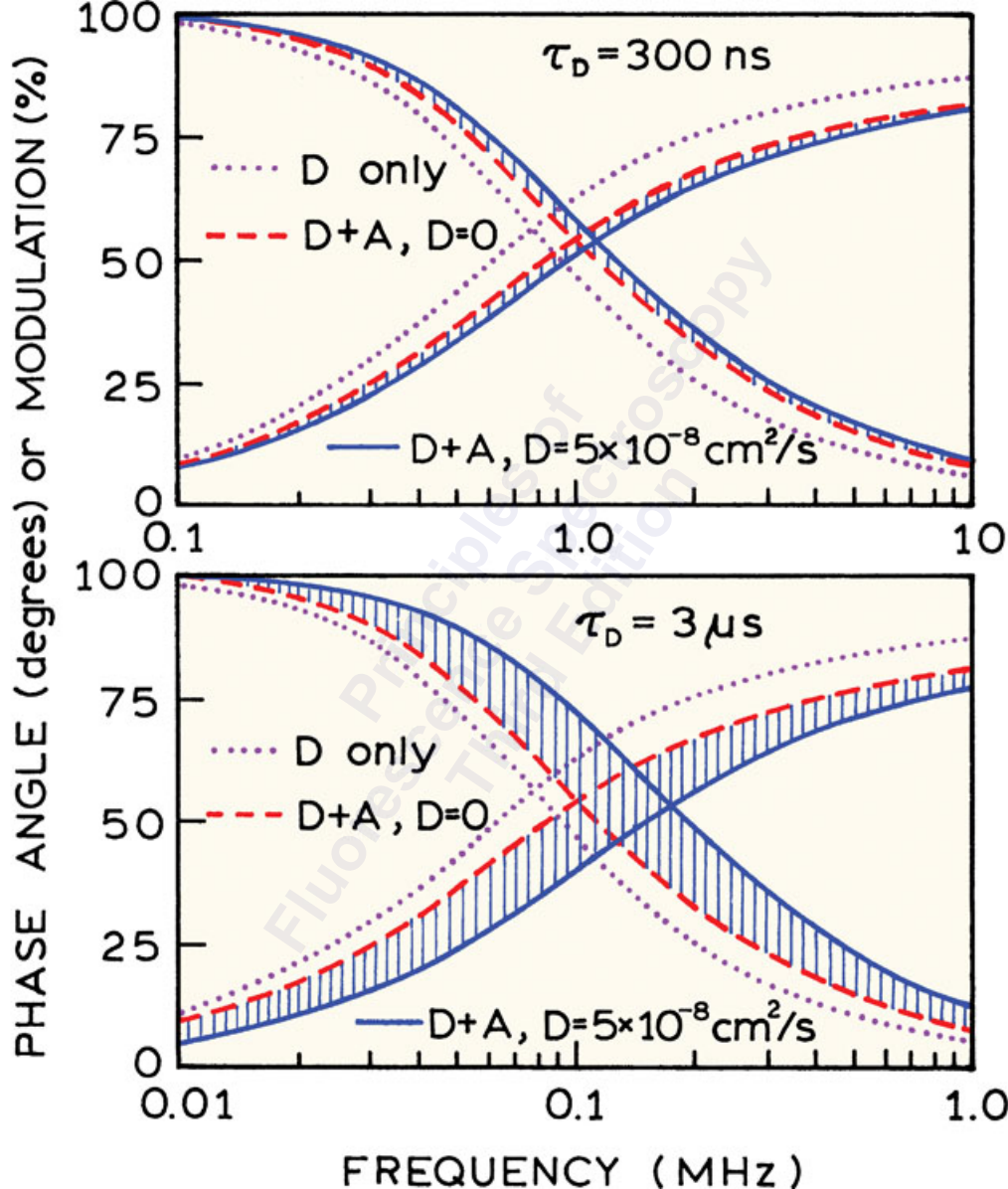


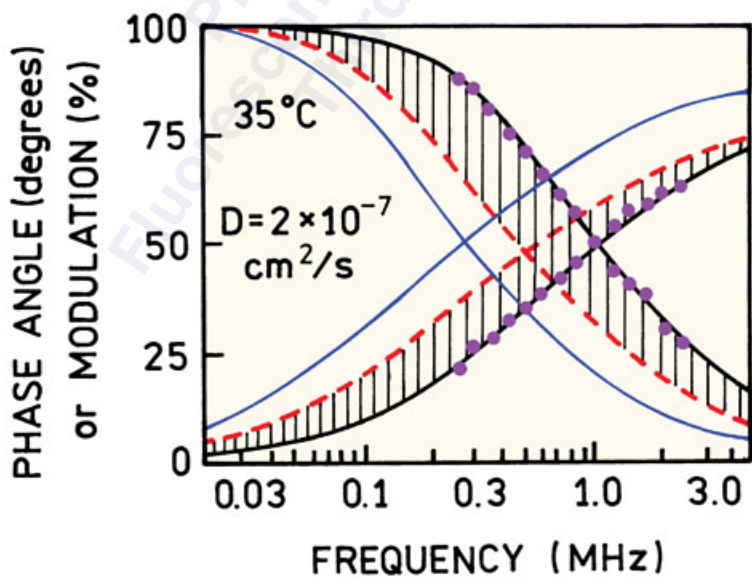
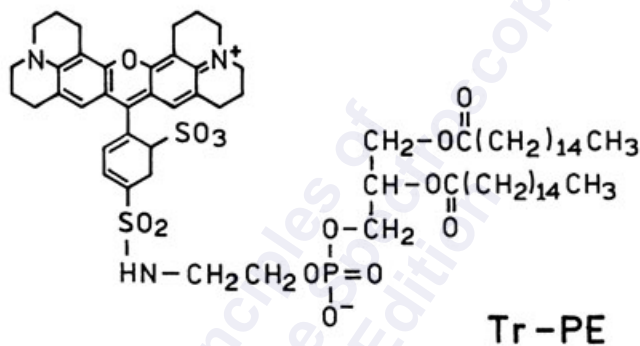
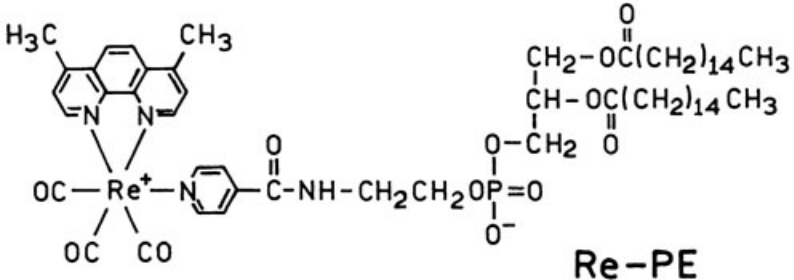












TRANSFER EFFICIENCY

1.0

1 s

0.5

1 ms

1  $\mu$ s

1 ns

0

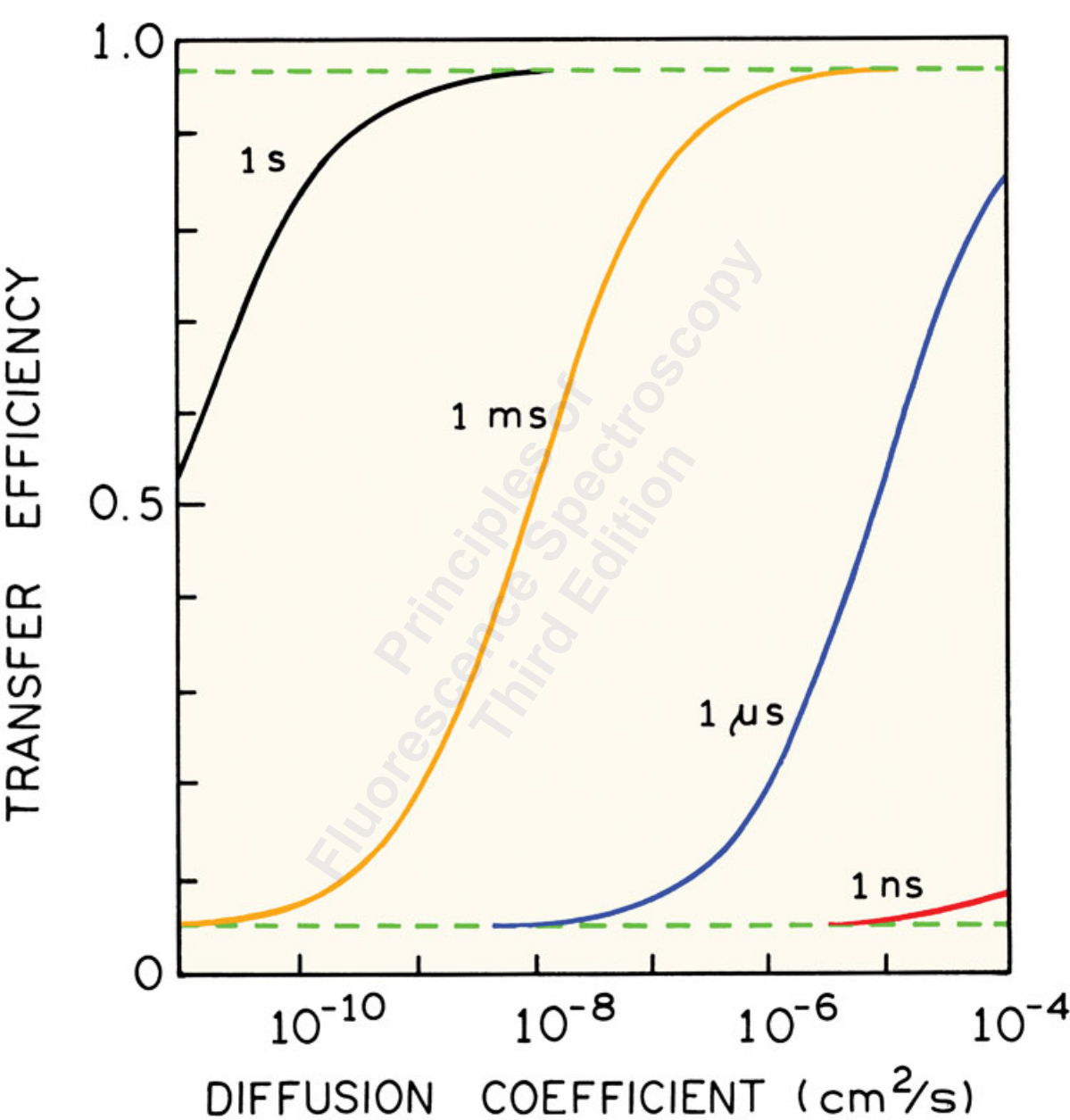
$10^{-10}$

$10^{-8}$

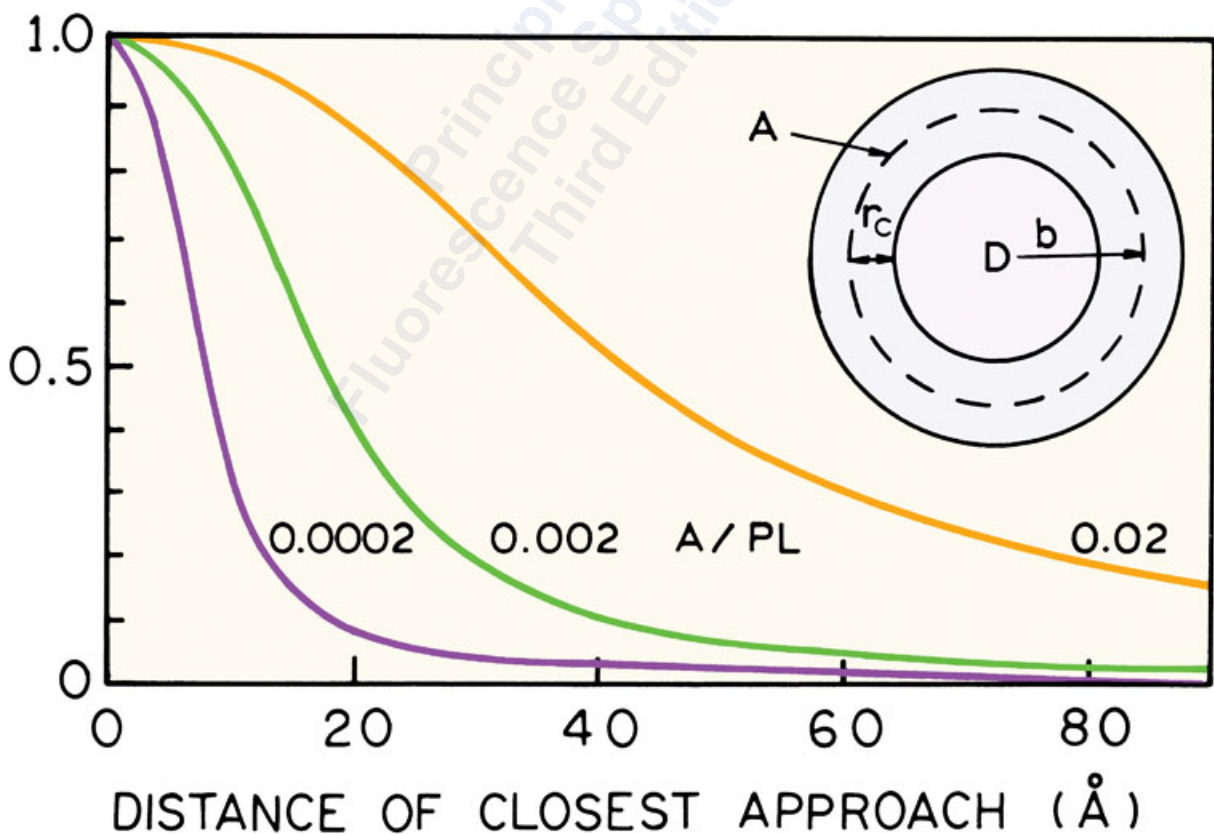
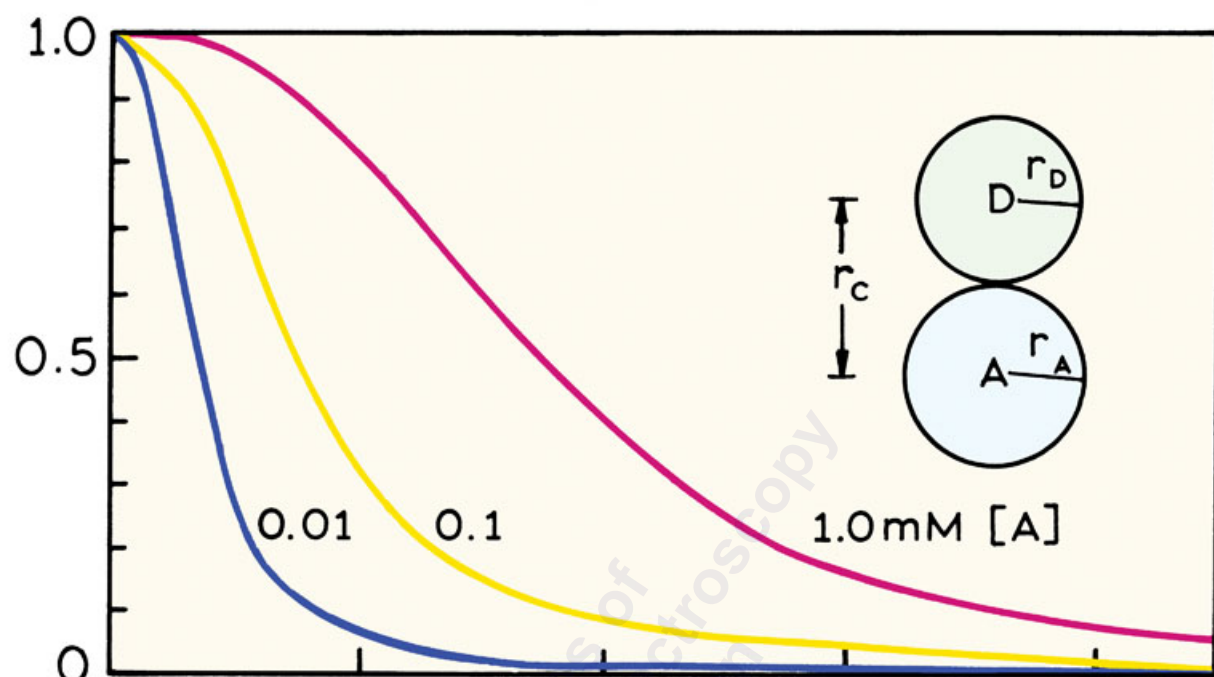
$10^{-6}$

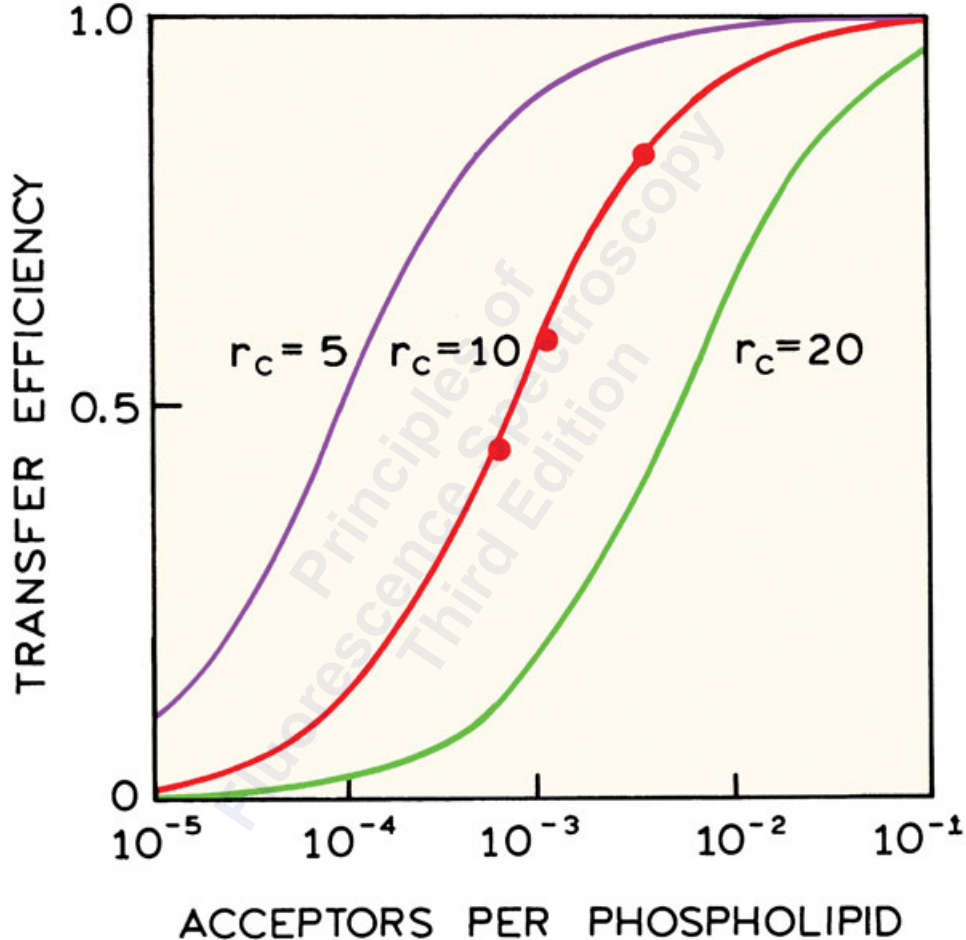
$10^{-4}$

DIFFUSION COEFFICIENT ( $\text{cm}^2/\text{s}$ )

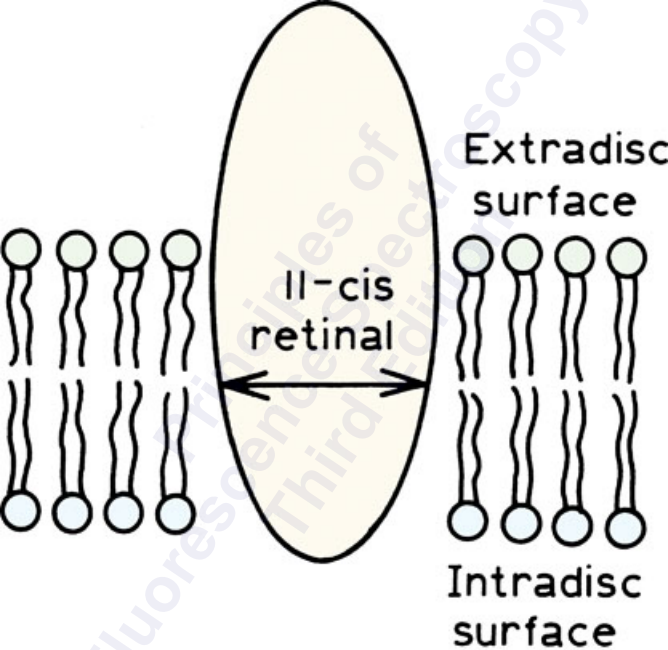


TRANSFER EFFICIENCY



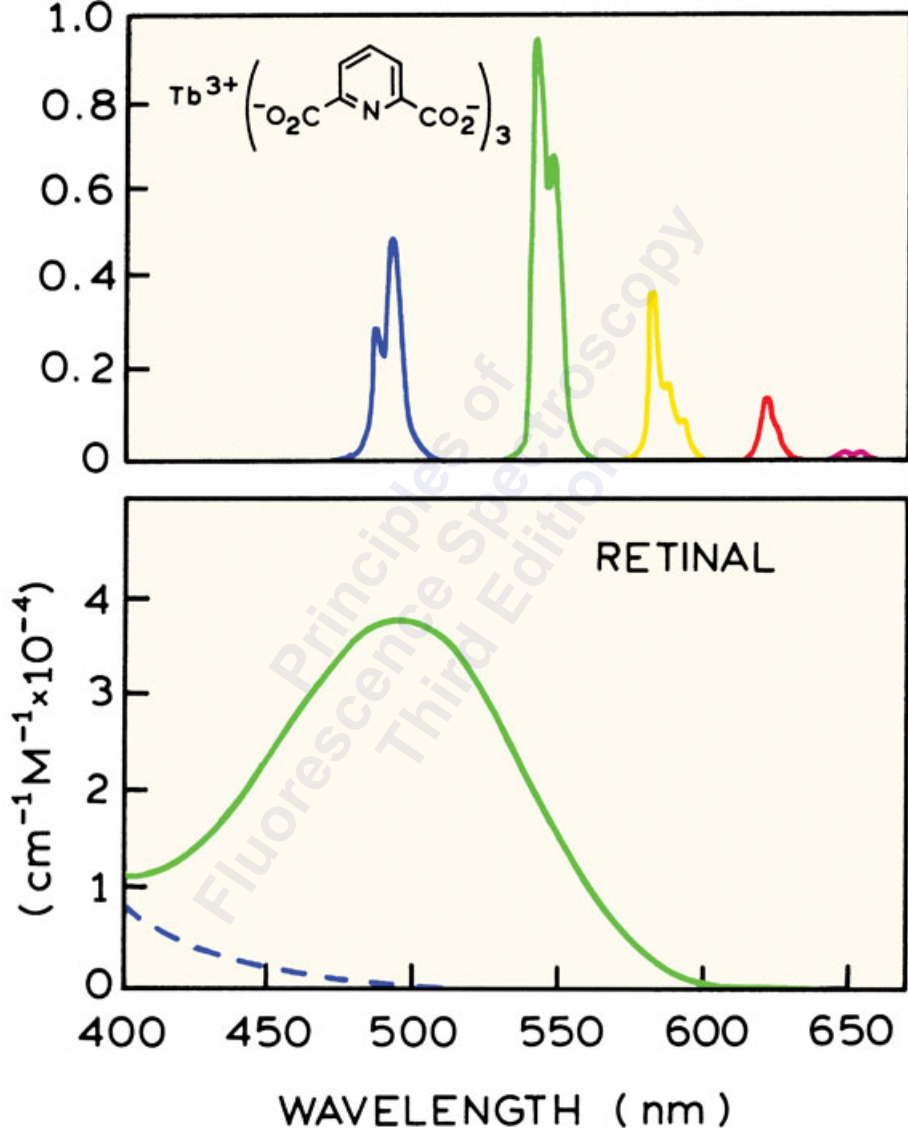


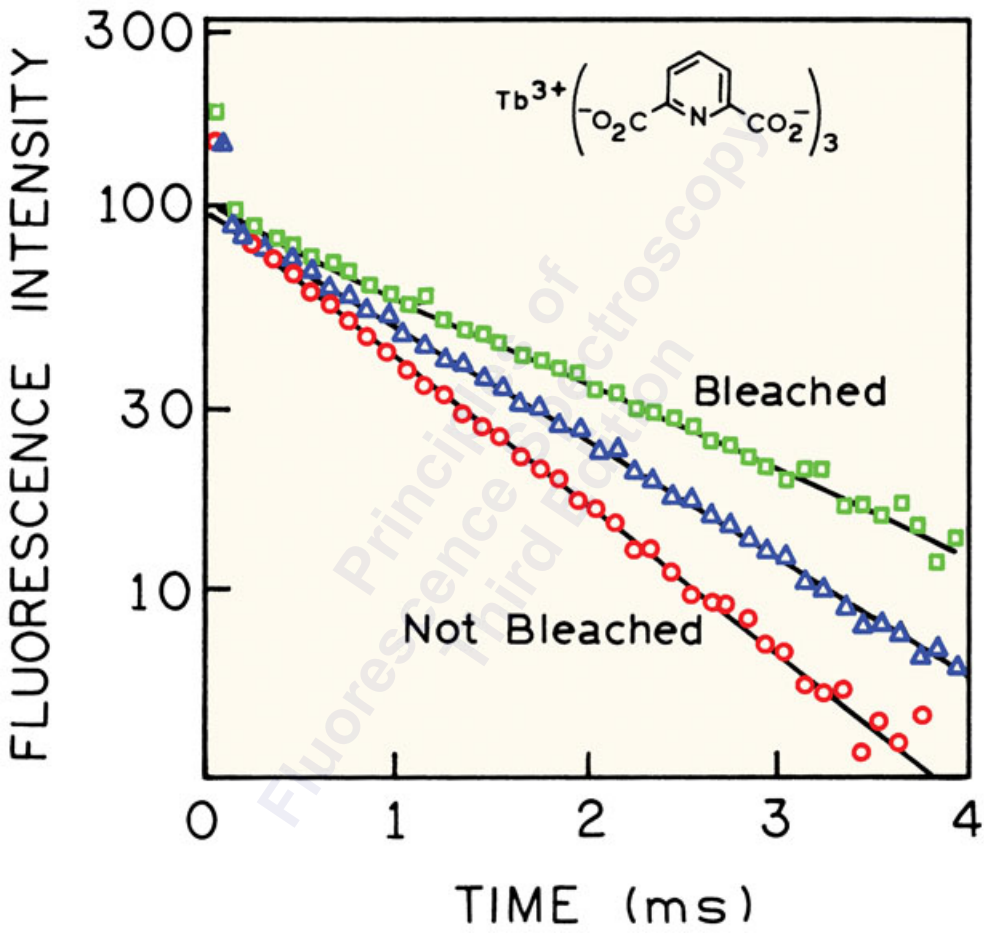




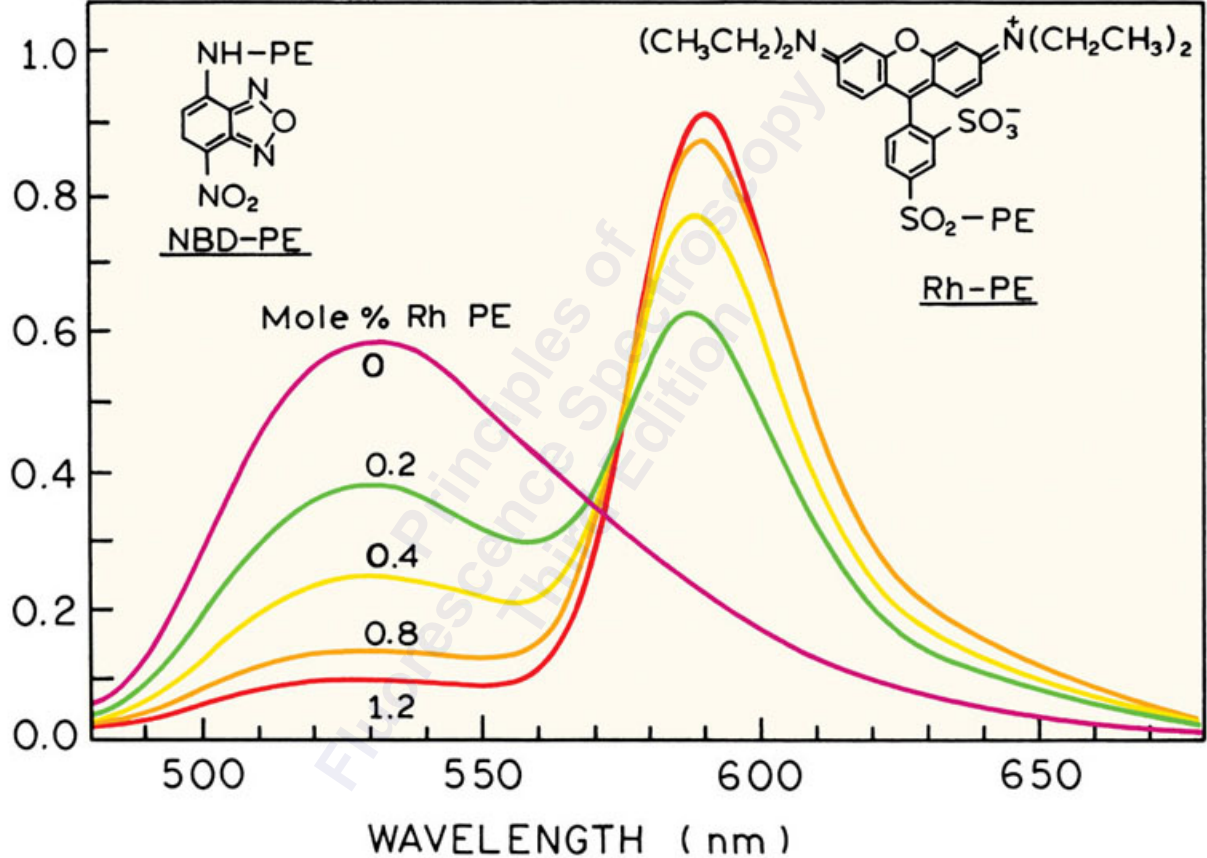


EXTINCTION COEFFICIENT FLUORESCENCE INT.

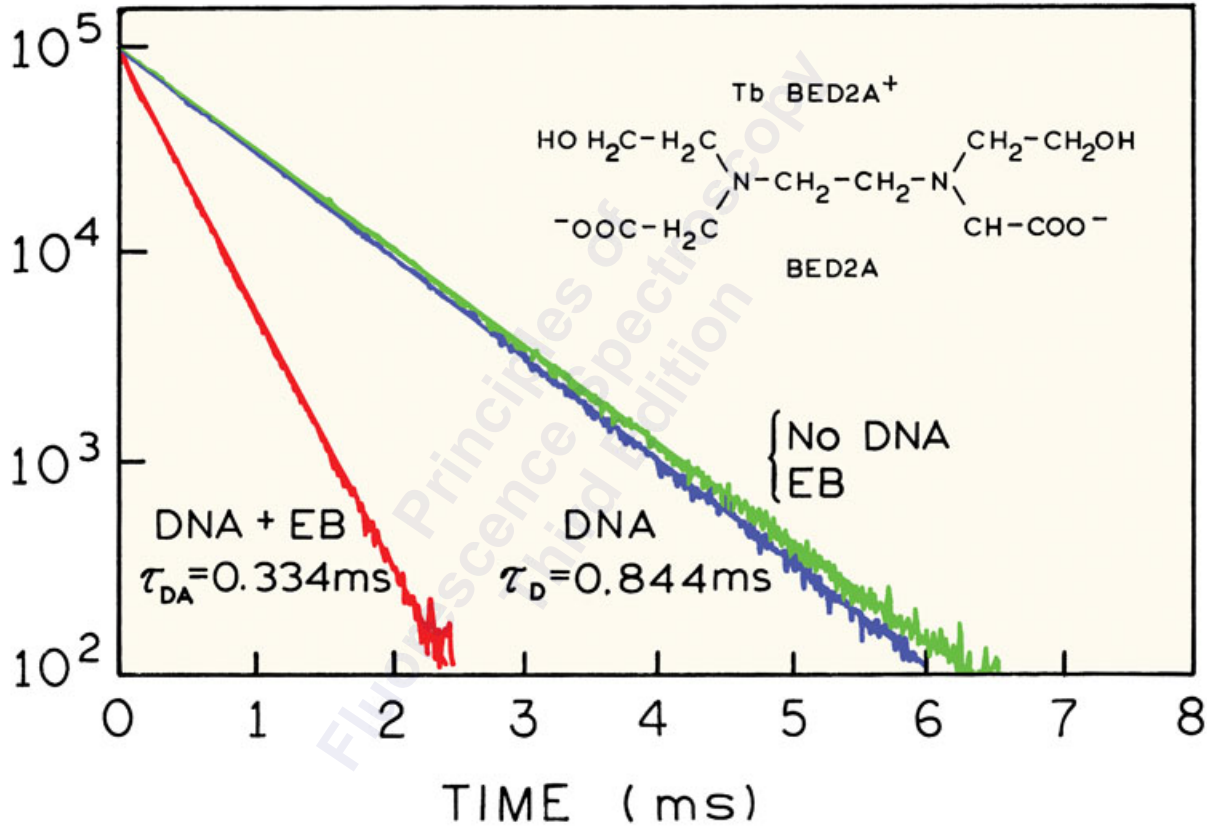


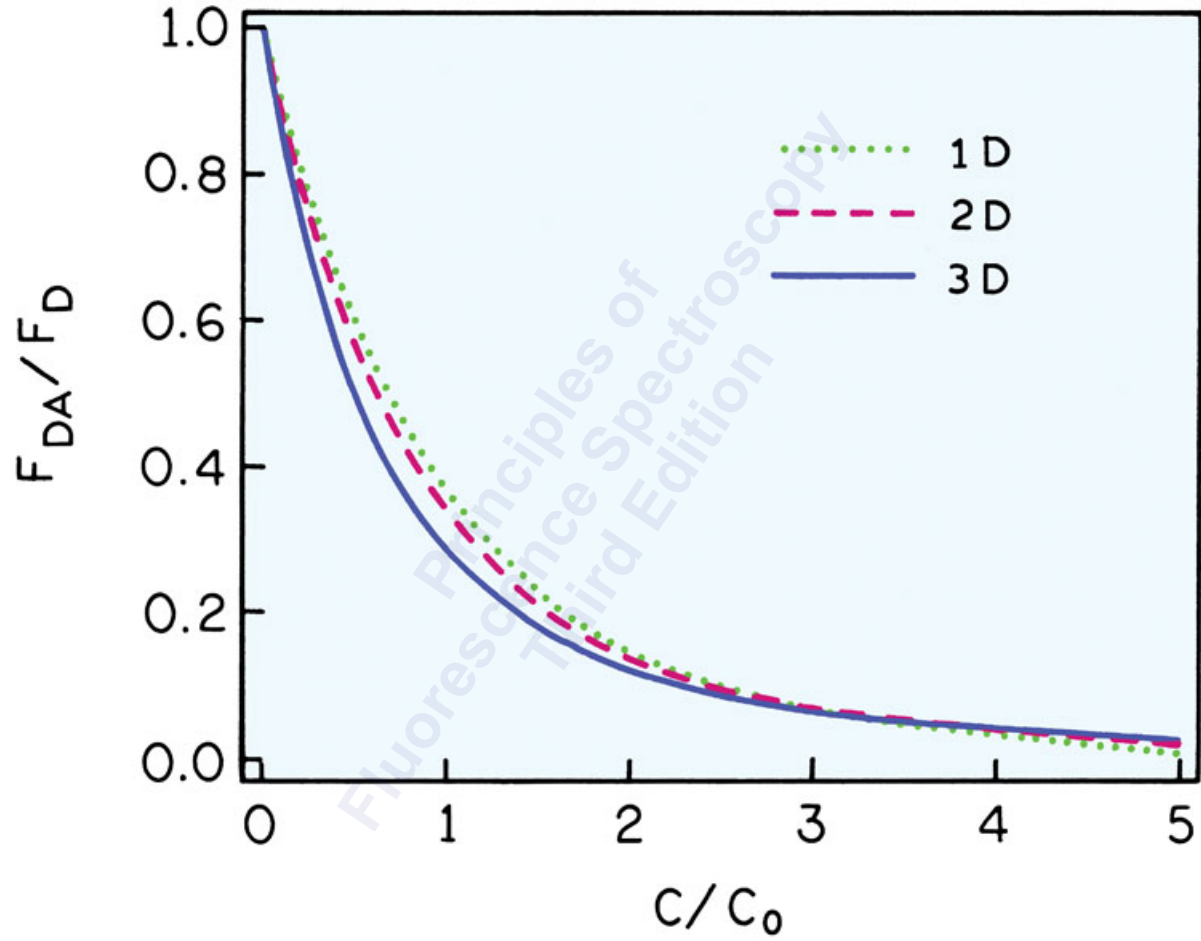


FLUORESCENCE INTENSITY

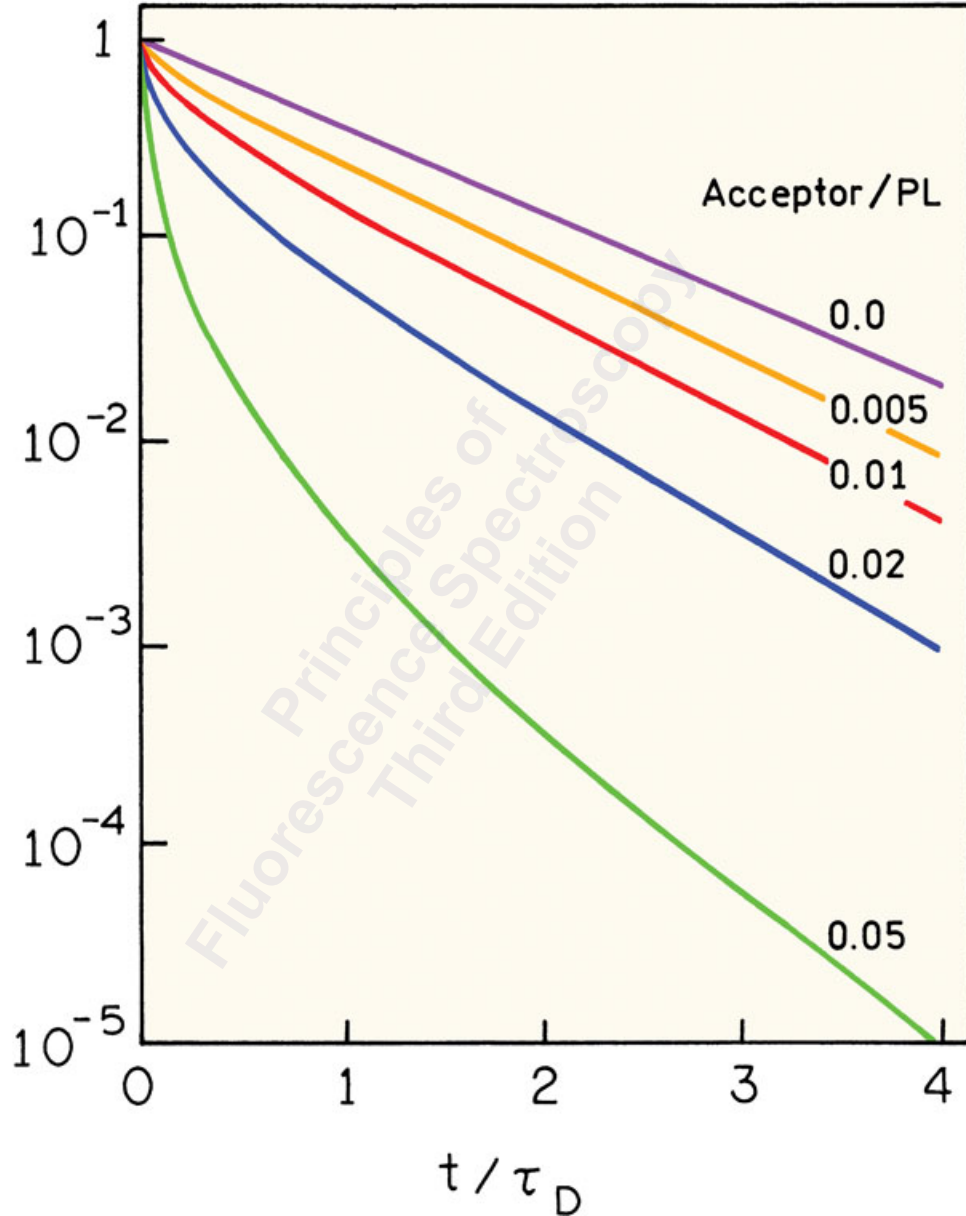


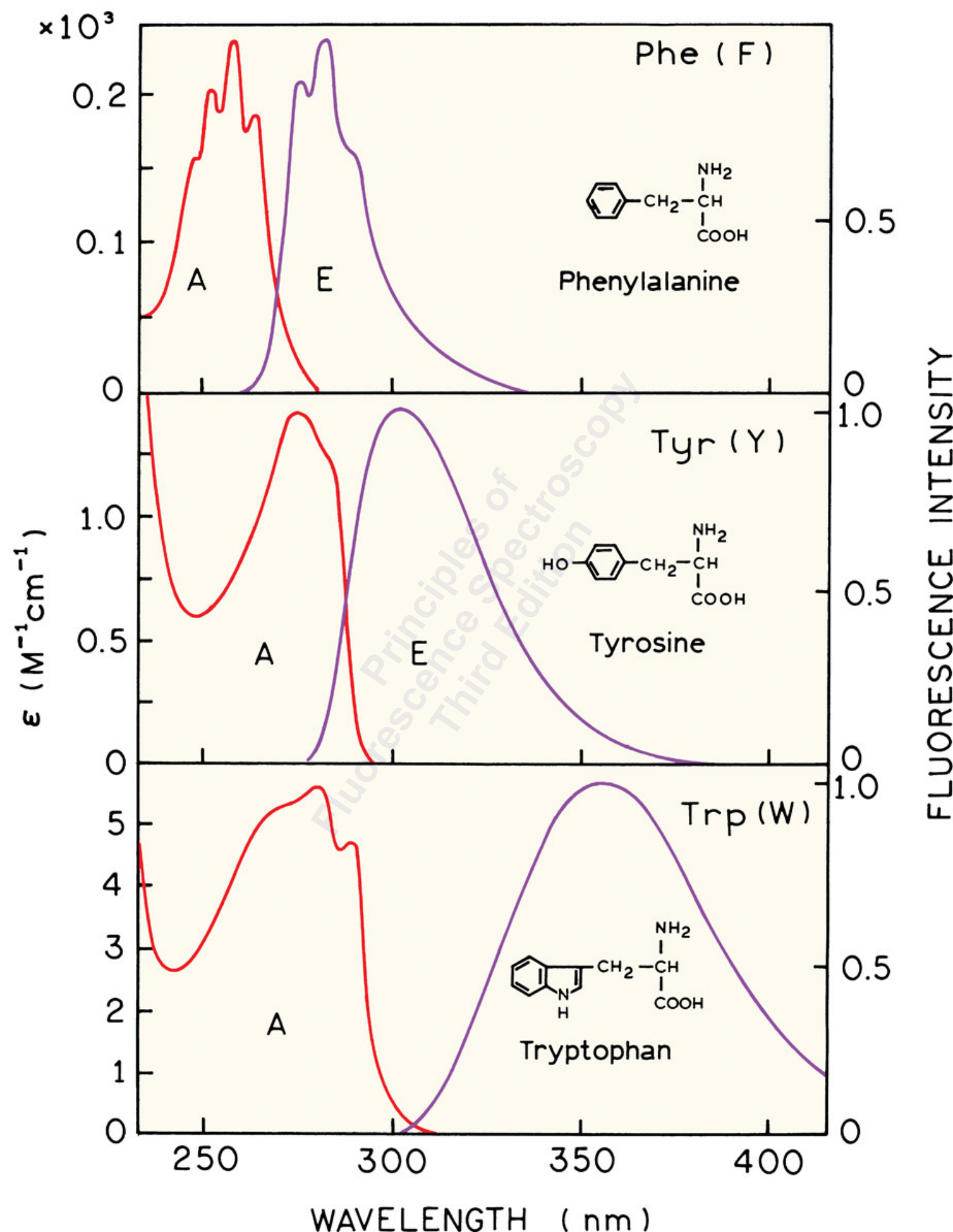
INTENSITY

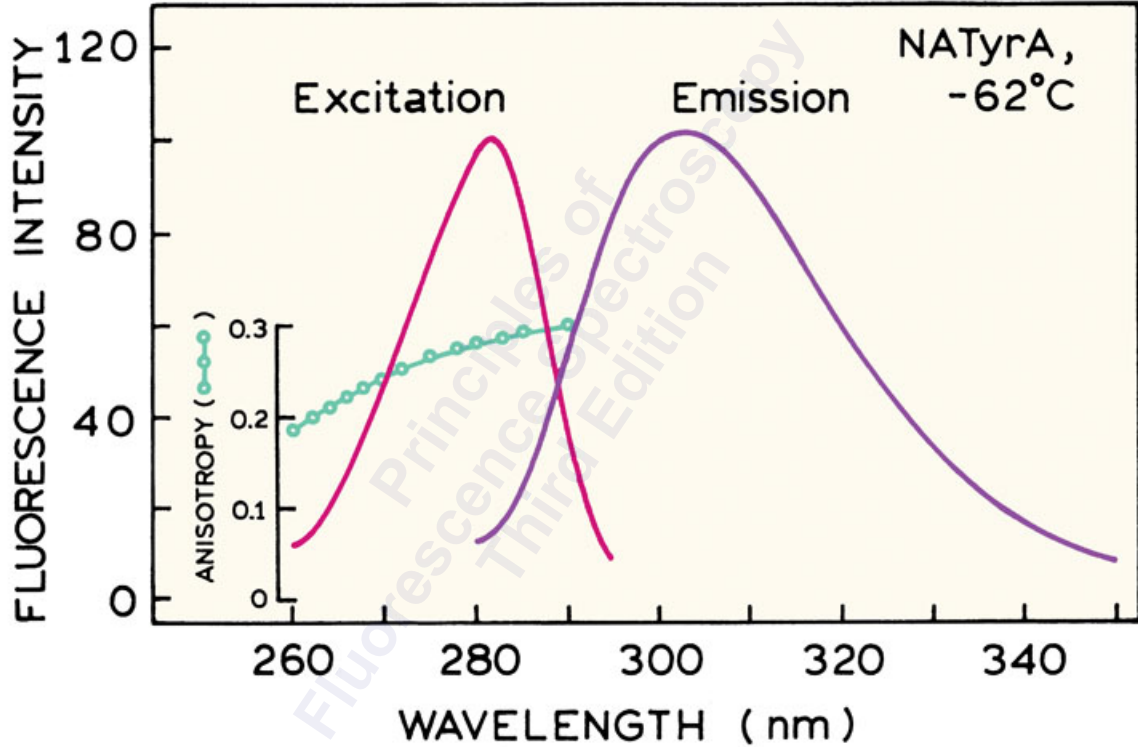




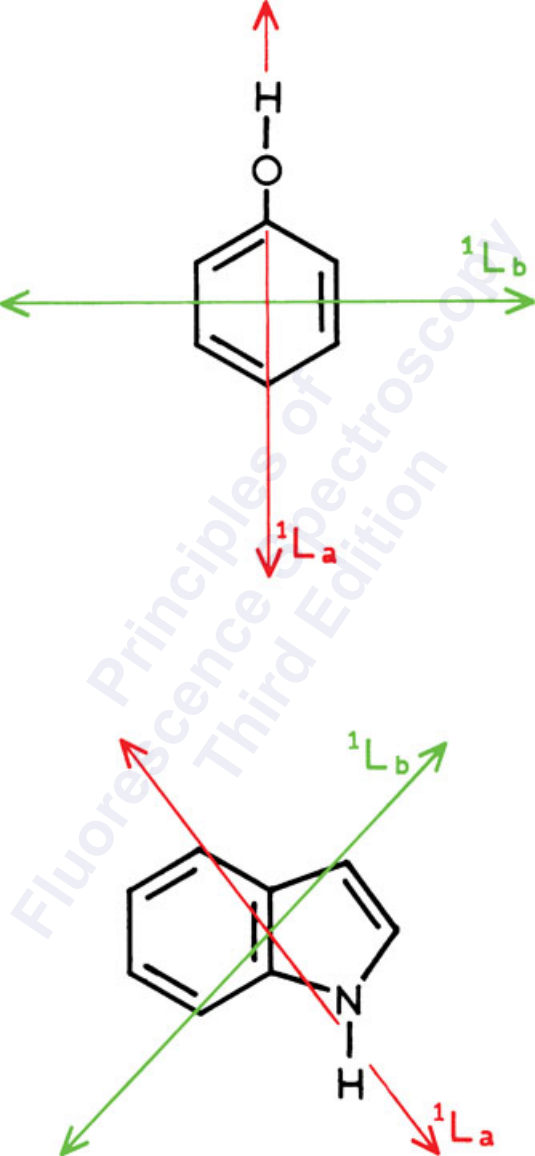
RELATIVE FLUORESCENCE INTENSITY

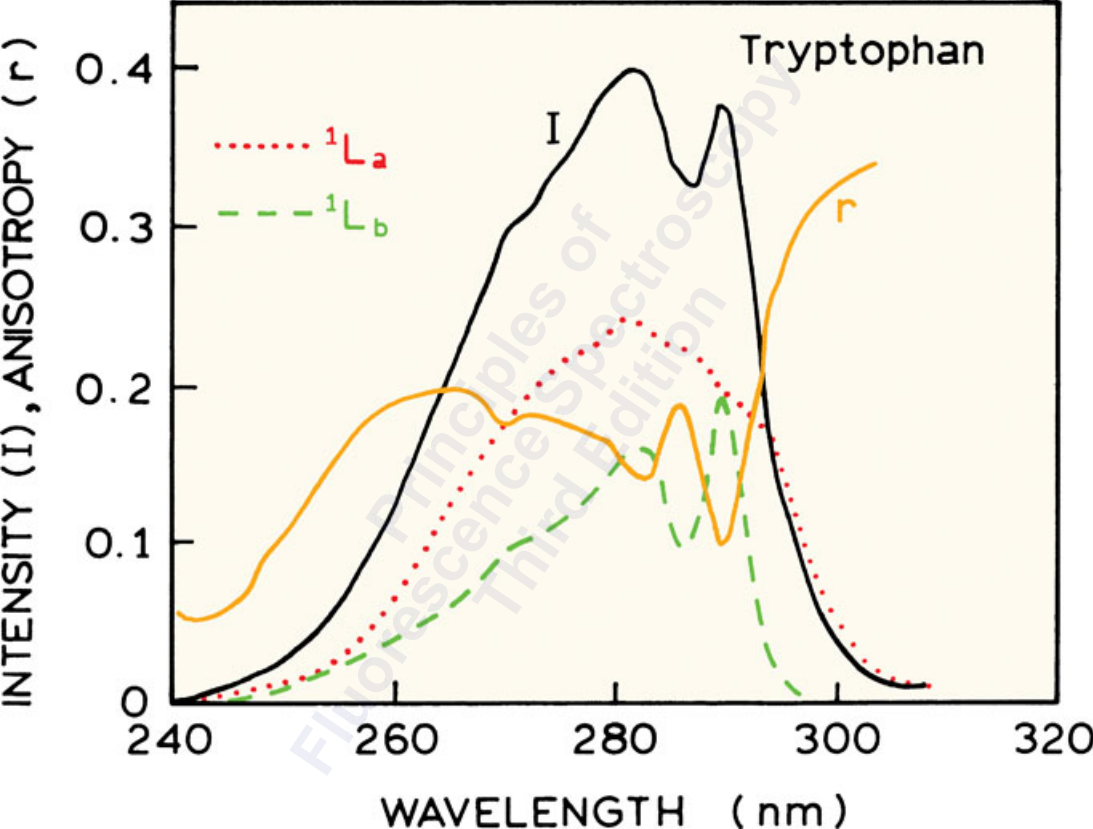


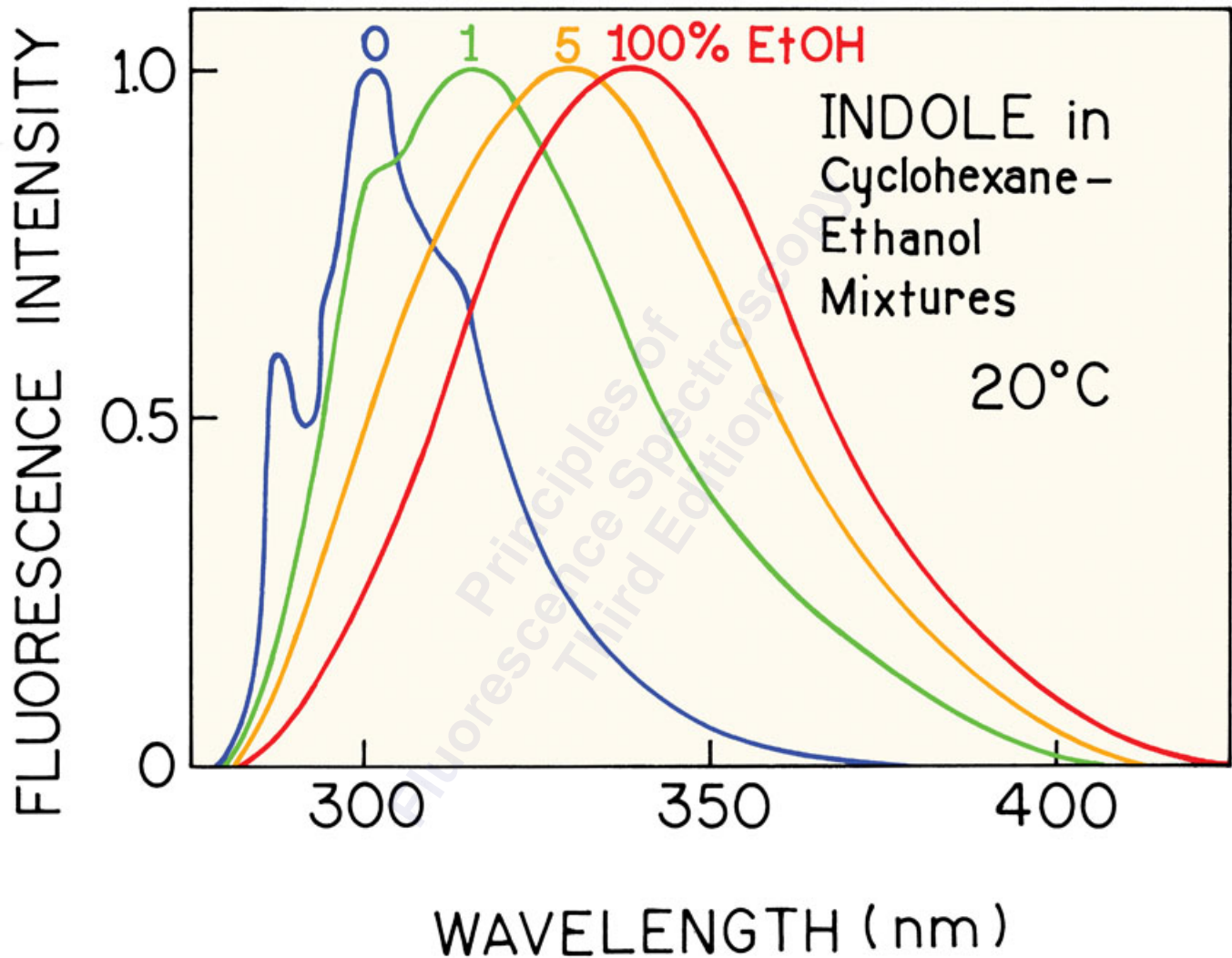


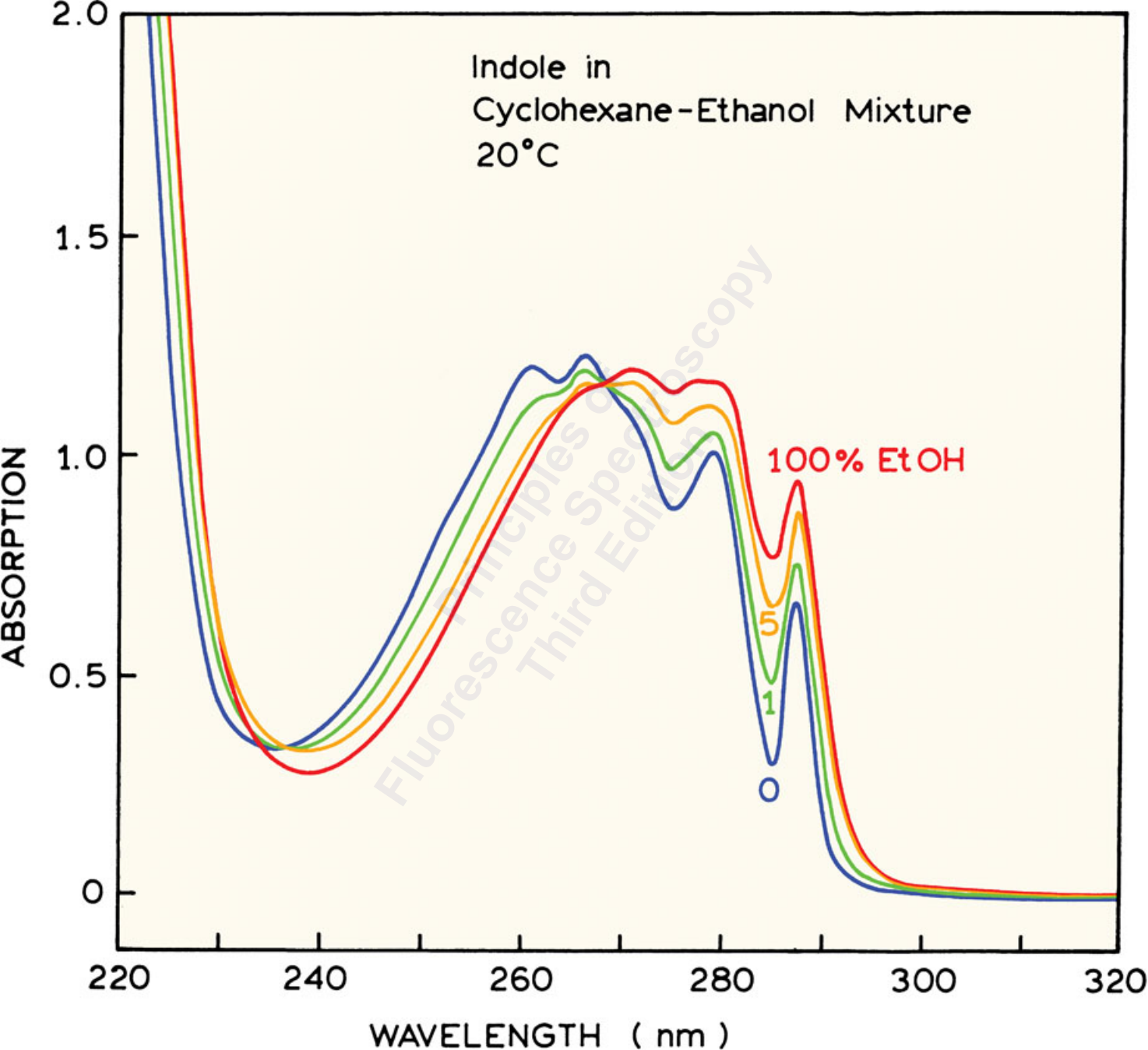


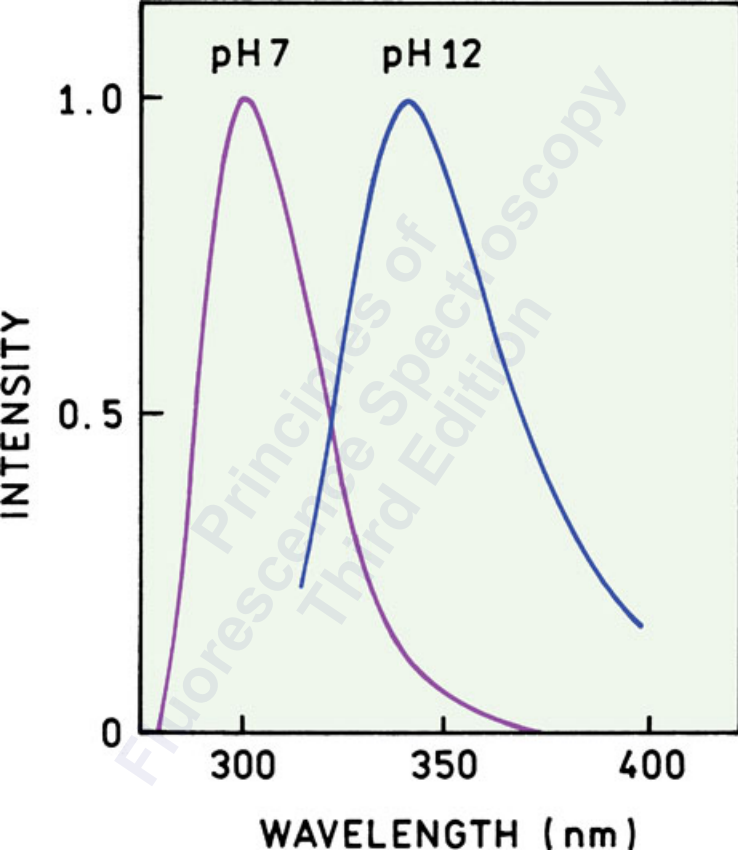






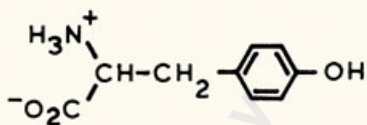






RELATIVE FLUORESCENCE INTENSITY

0.009 M



0.09 M

0.3 M

0.9 M

2.0 M

280

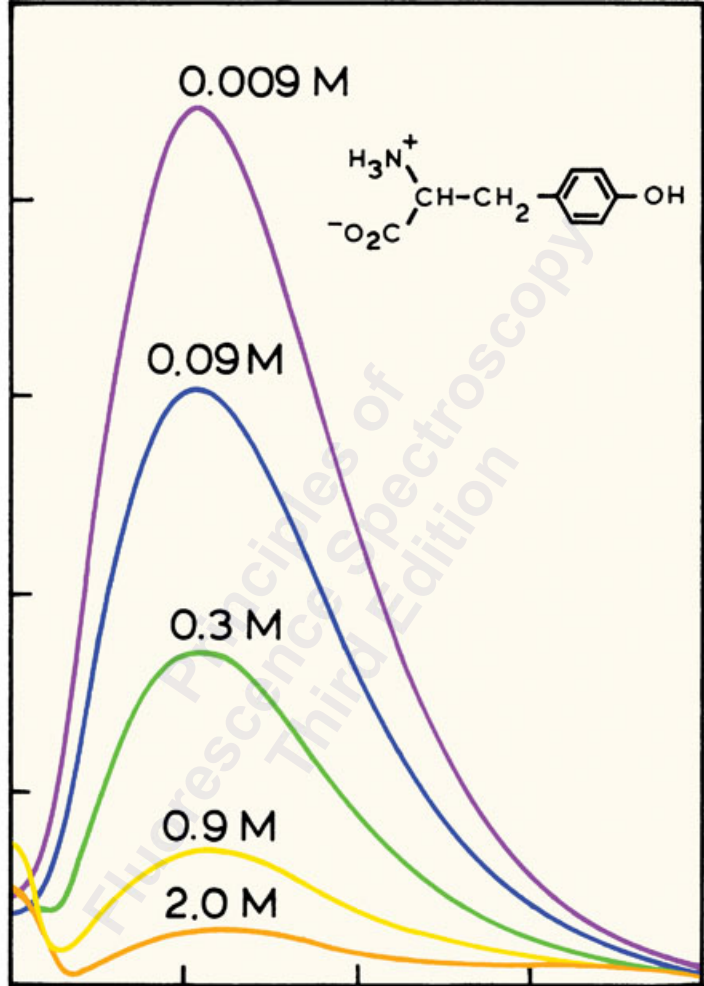
300

320

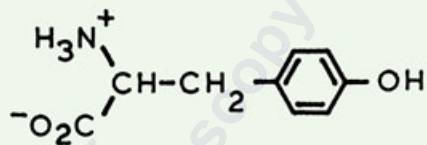
340

360

WAVELENGTH (nm)



NORMALIZED FLUORESCENCE INTENSITY



0.009 M

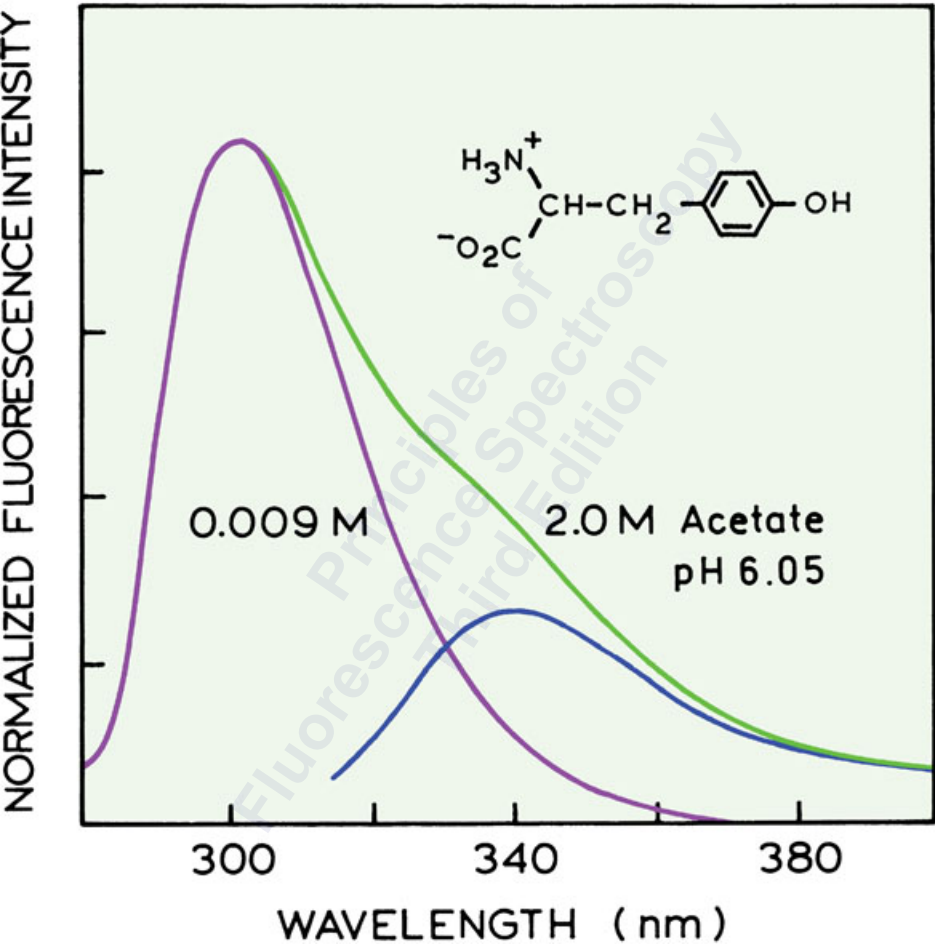
2.0 M Acetate  
pH 6.05

300

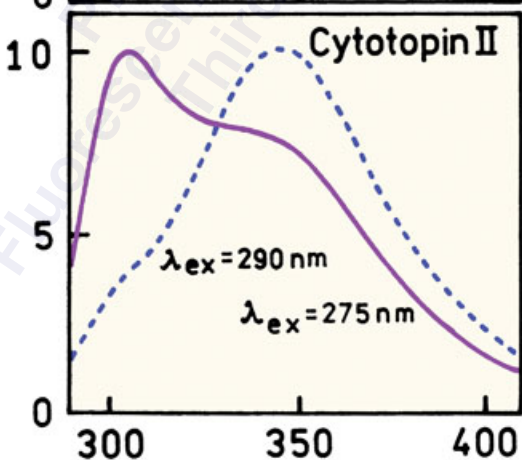
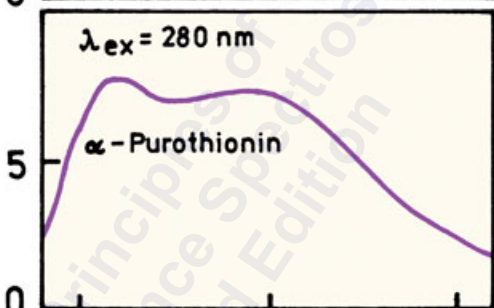
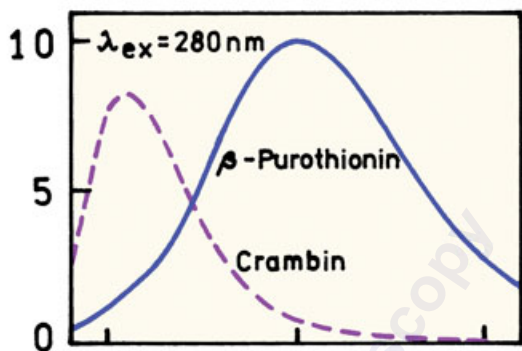
340

380

WAVELENGTH (nm)

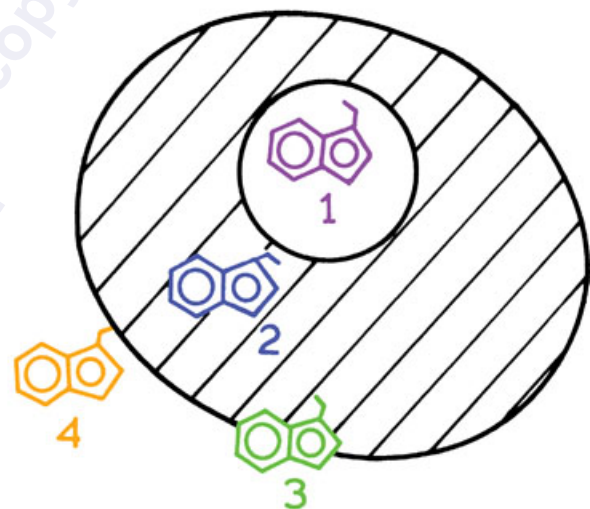
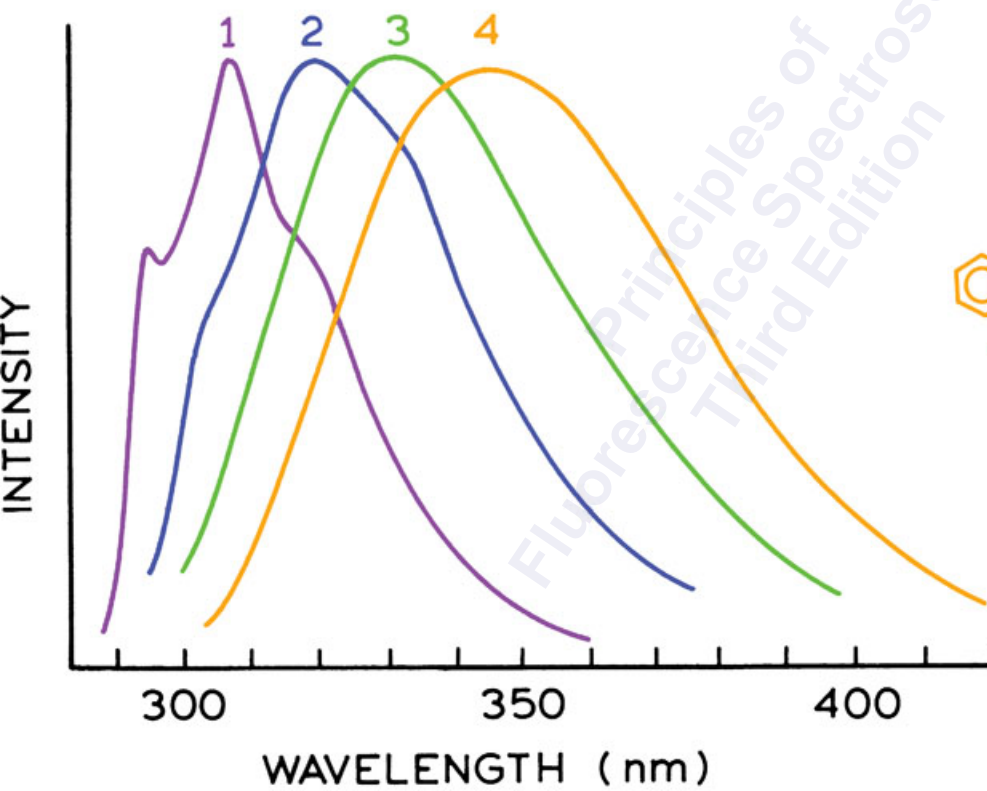
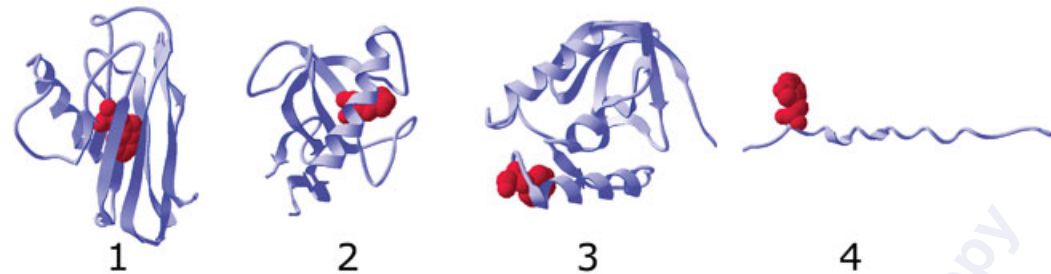


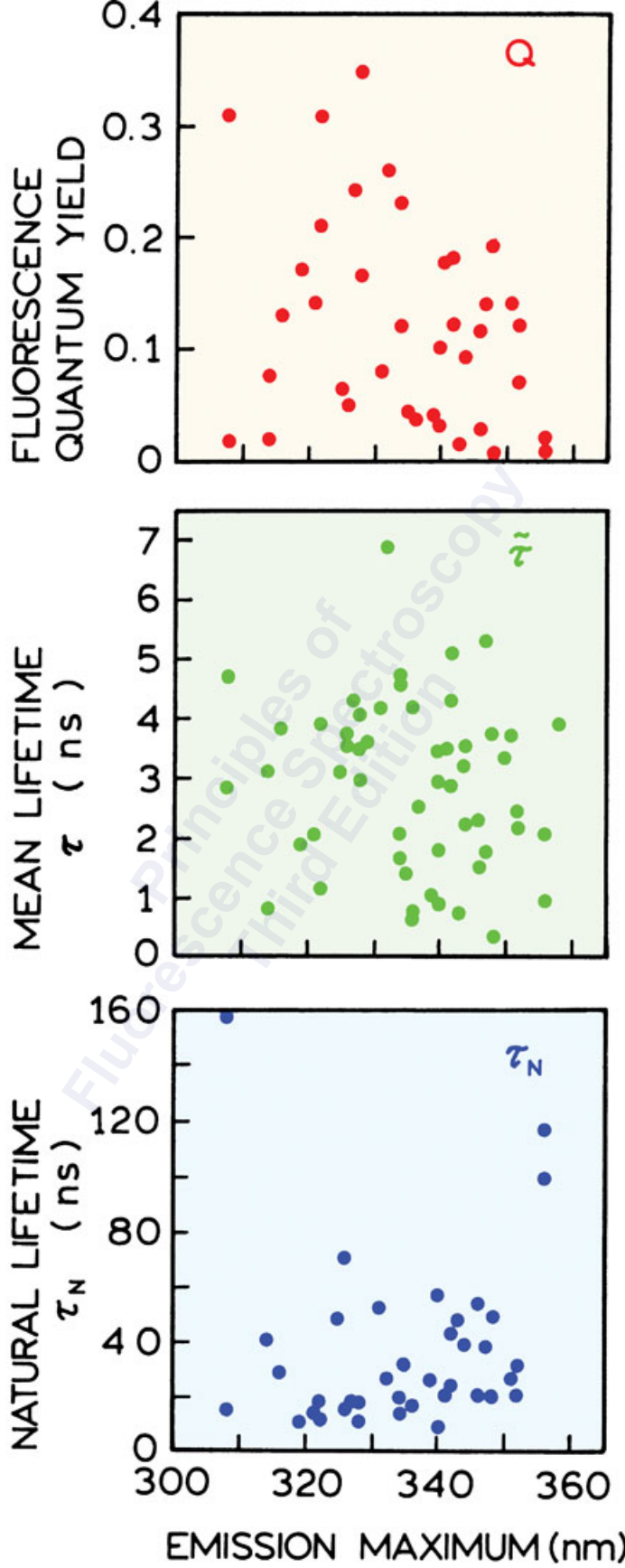
FLUORESCENCE INTENSITY (a.u.)

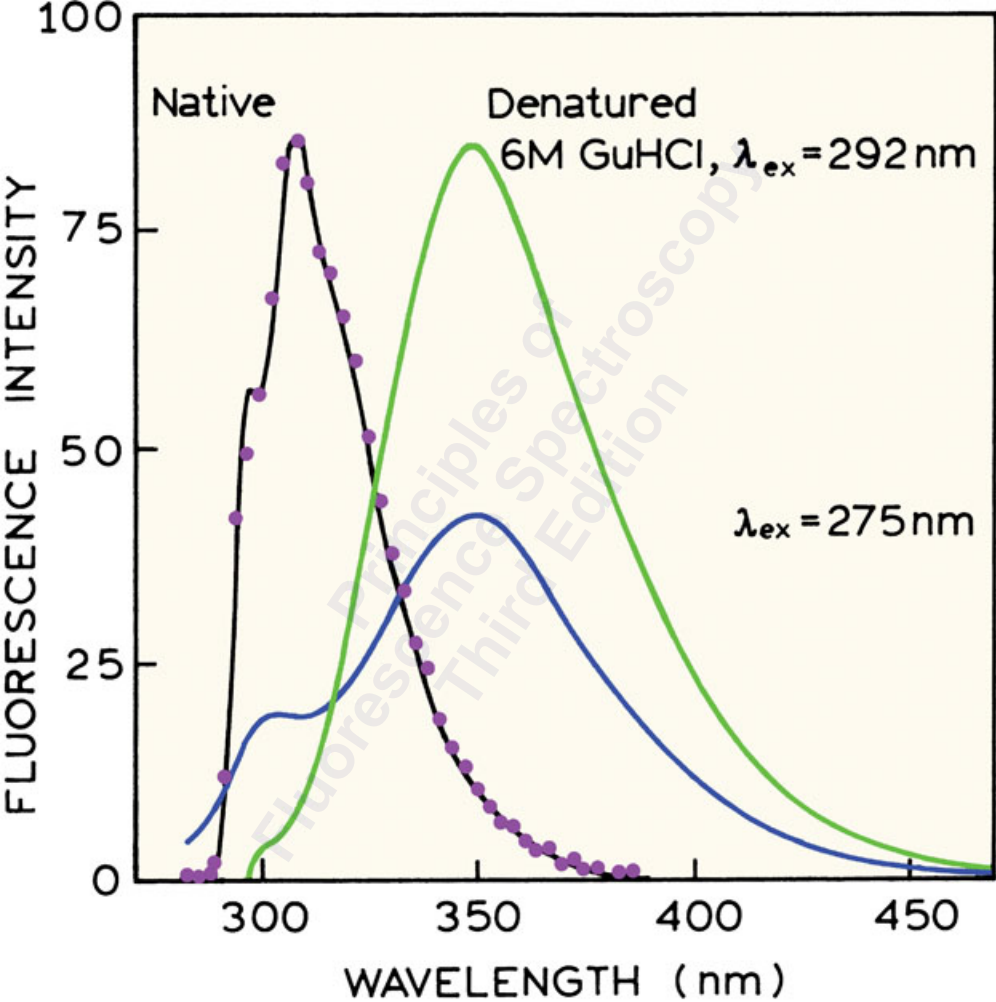


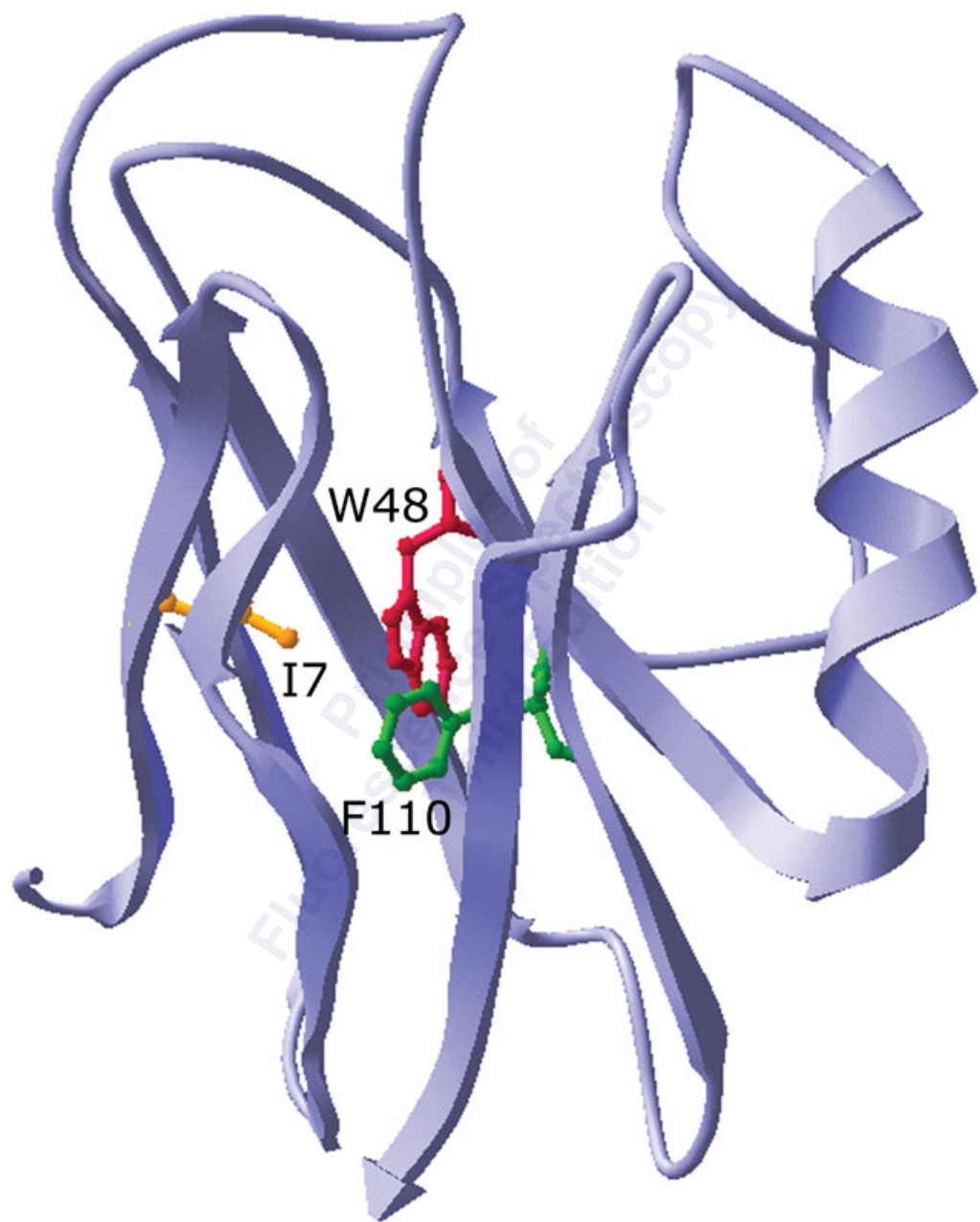
WAVELENGTH (nm)



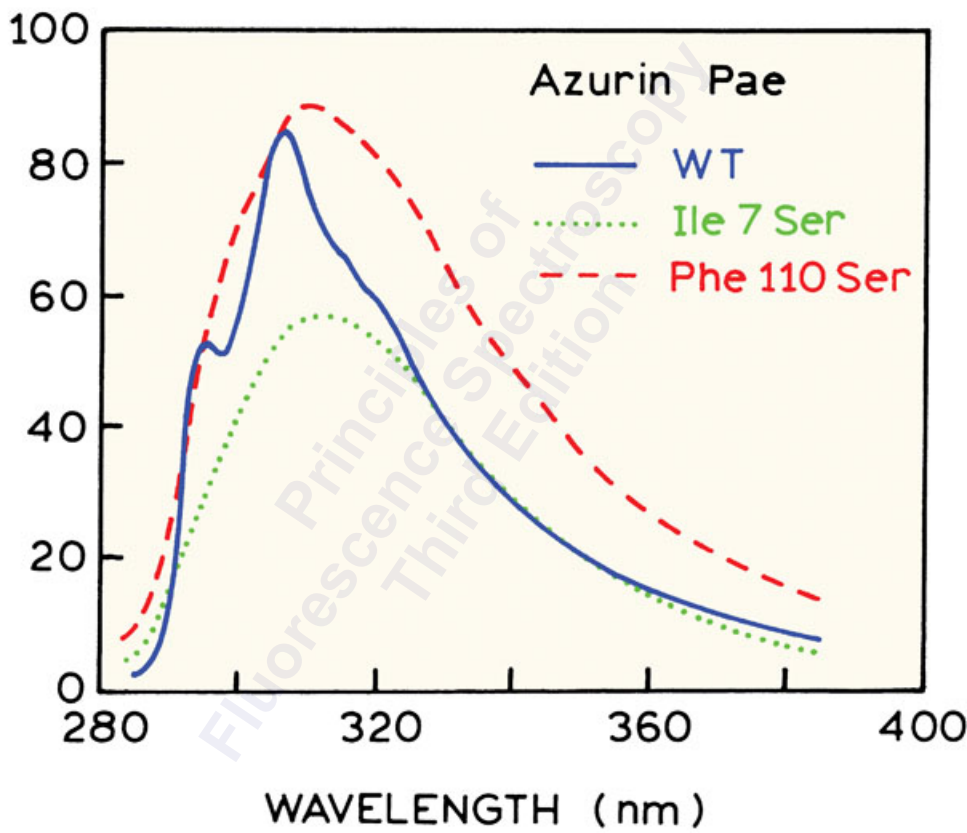


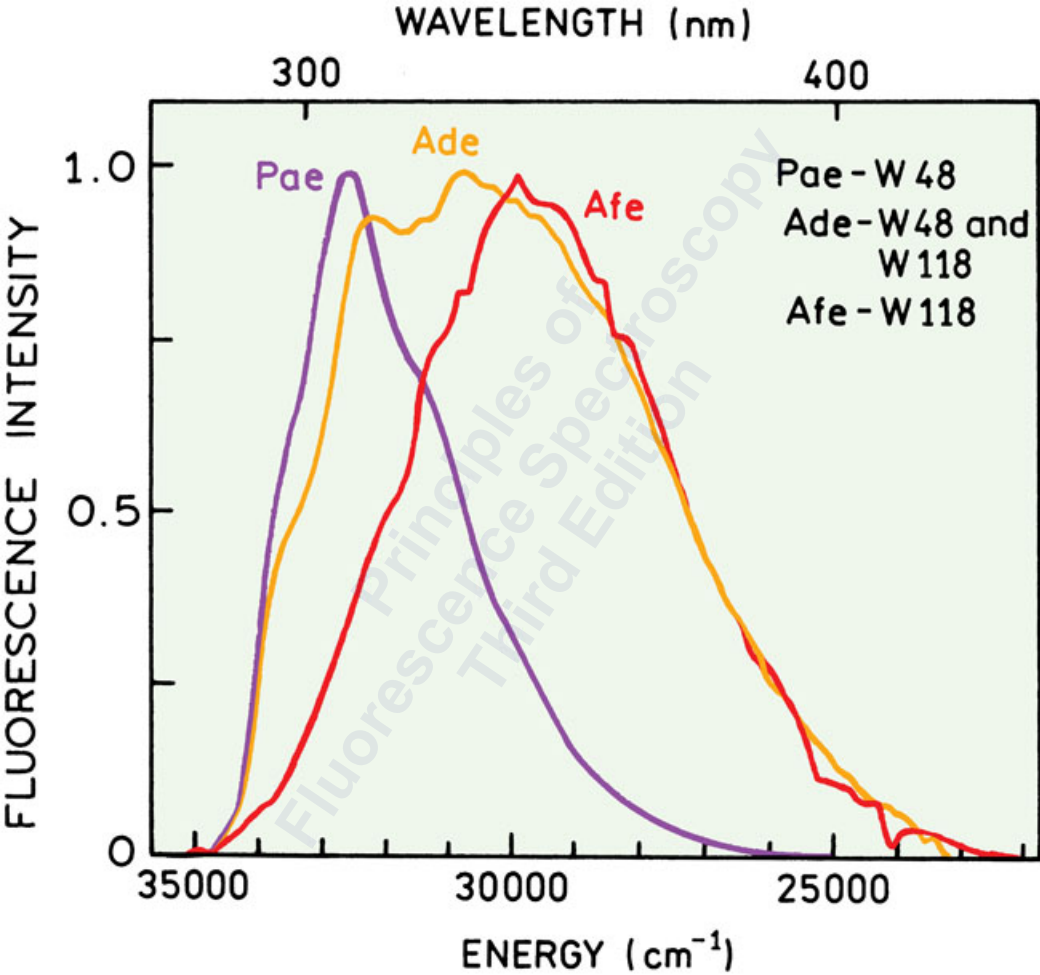


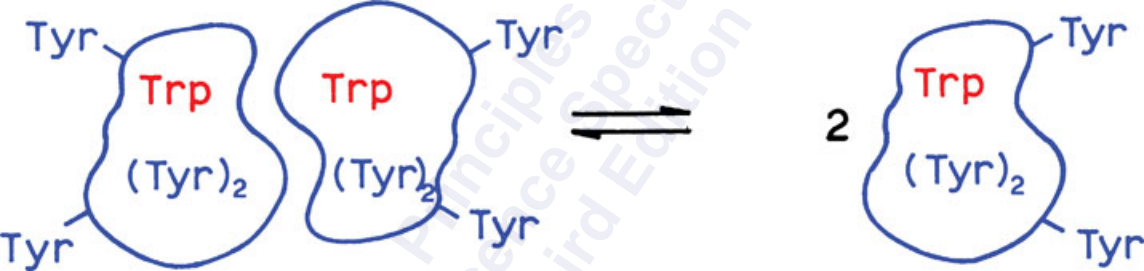


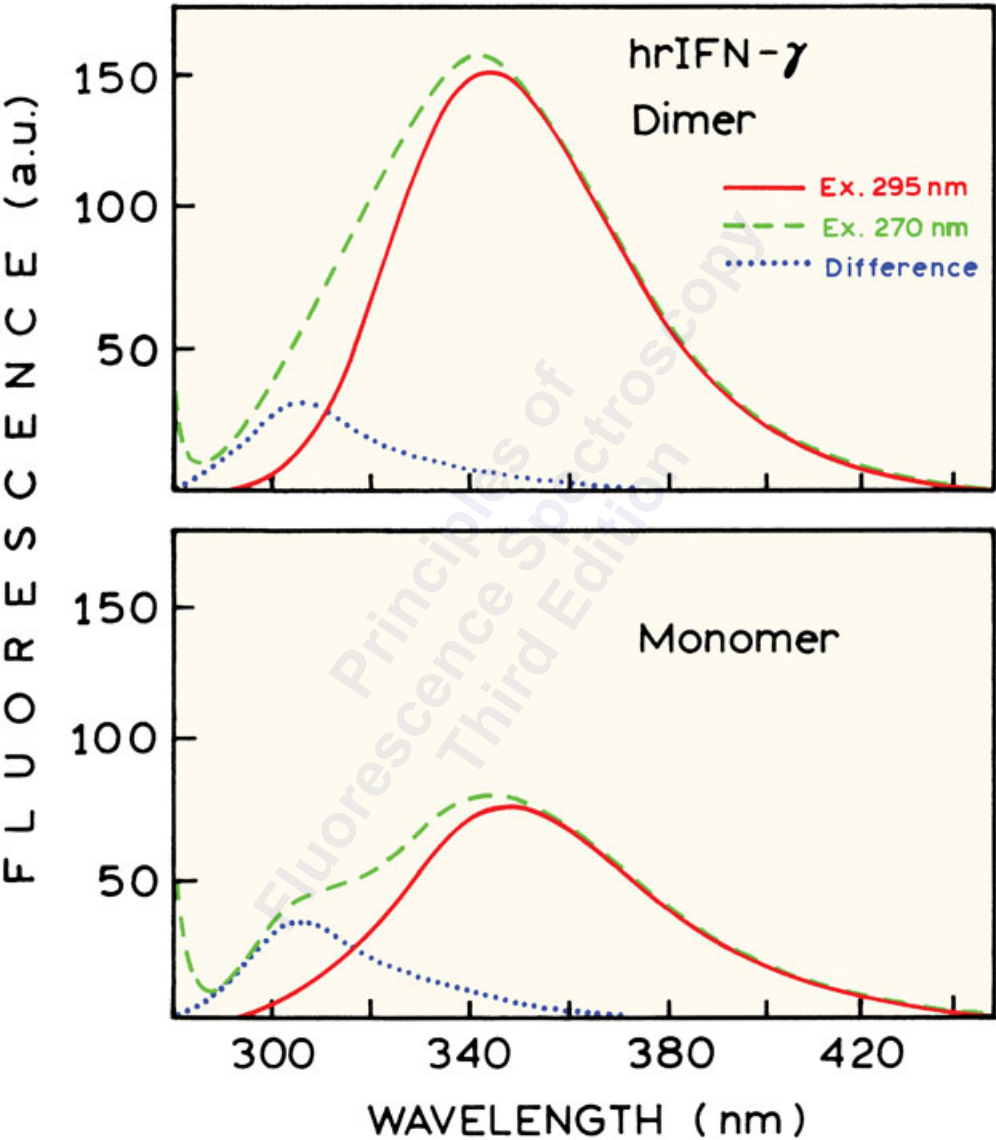


FLUORESCENCE











FRACTIONAL ABSORBANCE

260

280

300

WAVELENGTH ( nm )

1.0

0.8

0.6

0.4

0.2

Y:W:P

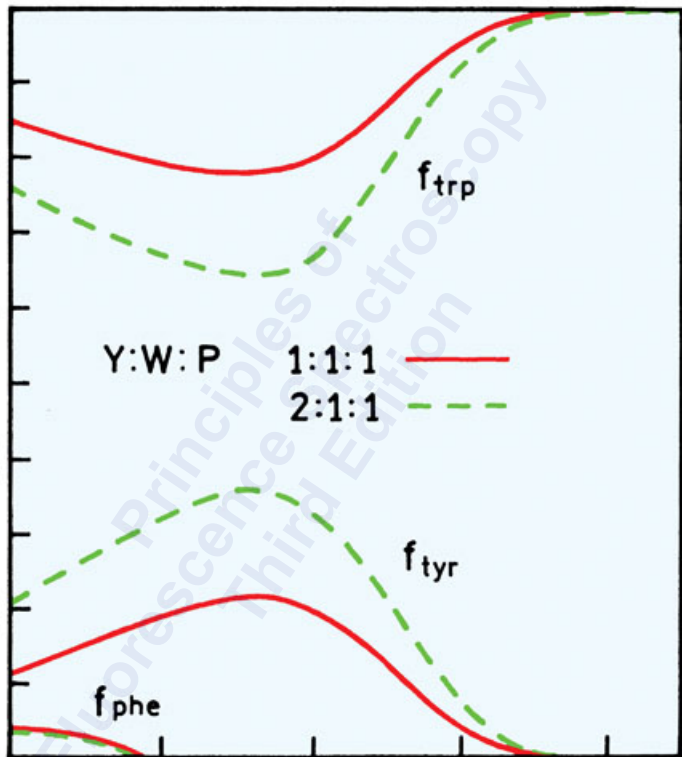
1:1:1

2:1:1

$f_{\text{trp}}$

$f_{\text{tyr}}$

$f_{\text{phe}}$



RELATIVE QUANTUM YIELD

Trp-Tyr

Trp+Tyr Mixture  
(1:1)

$E = 1.0$

$E = 0.5$

$E = 0$

1.0

0.9

0.8

0.7

260

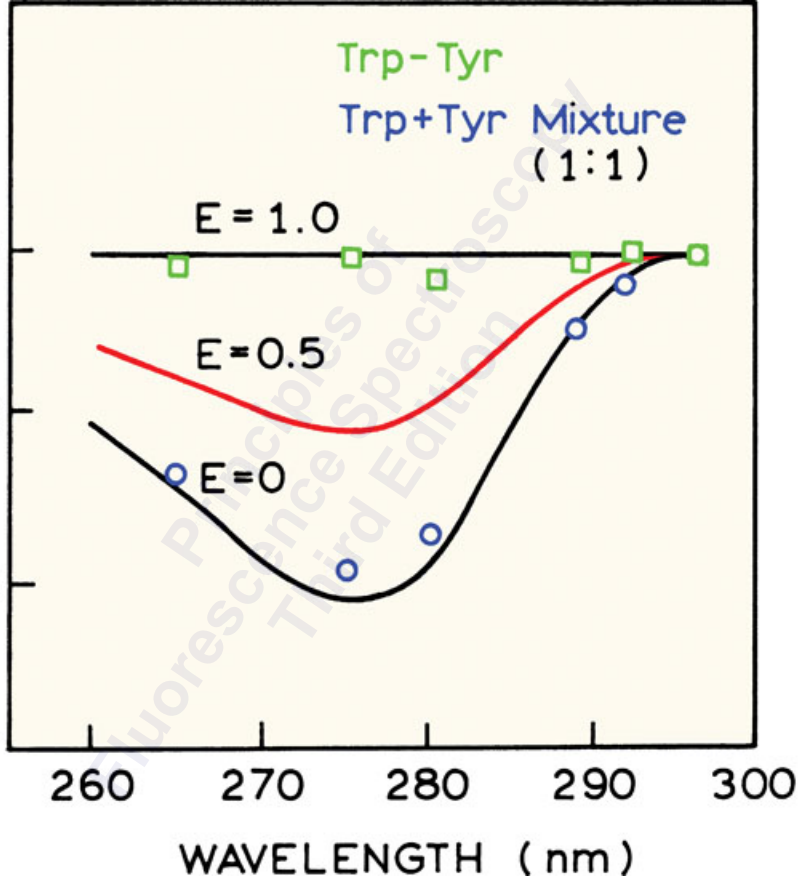
270

280

290

300

WAVELENGTH (nm)



RELATIVE QUANTUM YIELD  $Q(\lambda)$

hr IFN- $\gamma$

$E=1.0$

$E=0.7$

$E=0.6$

DIMER

MONOMER

$E=0.2$

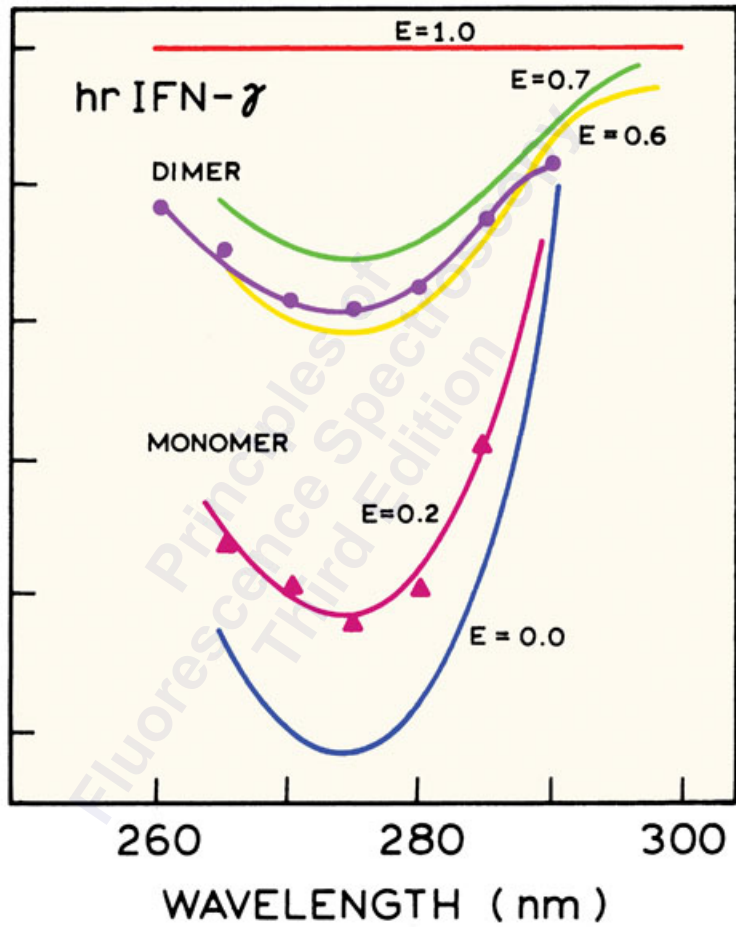
$E=0.0$

260

280

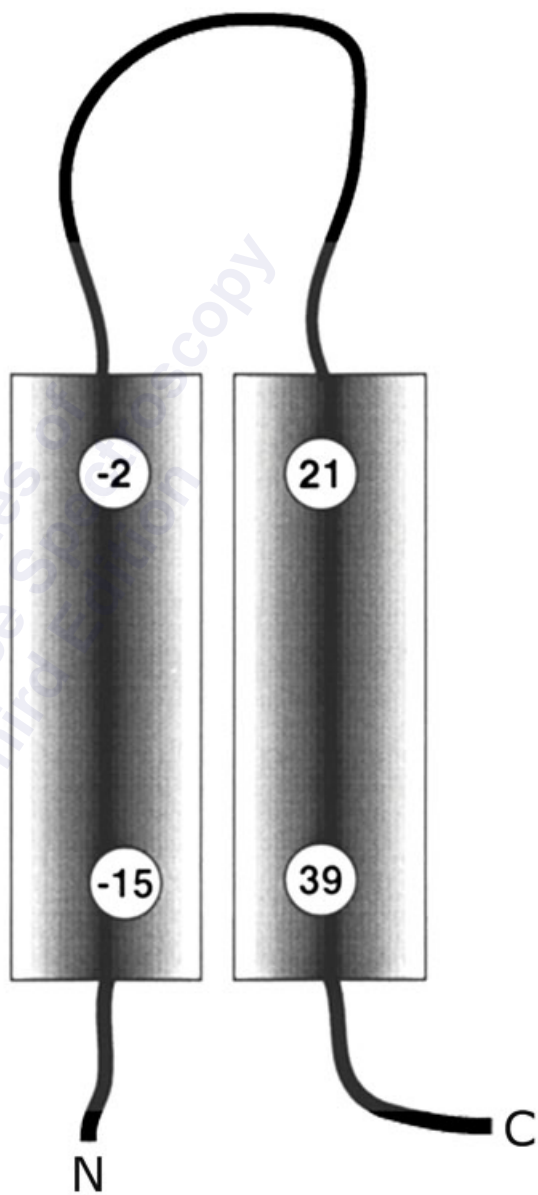
300

WAVELENGTH (nm)

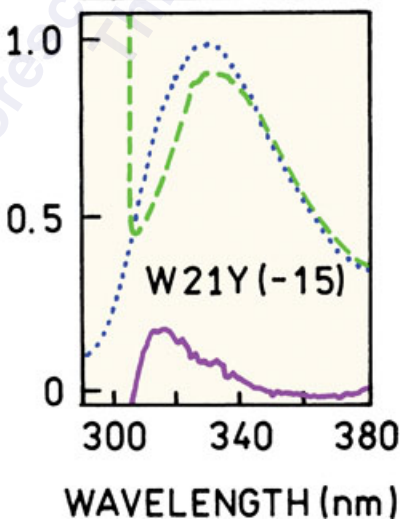
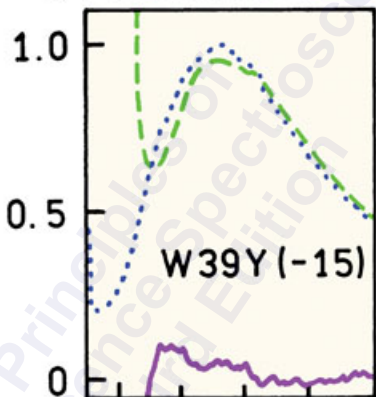
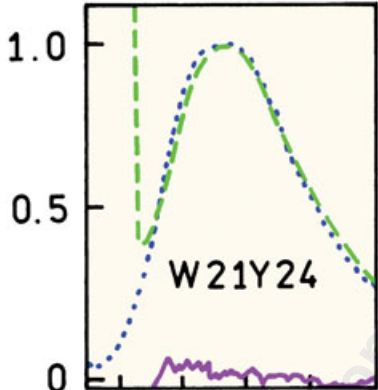


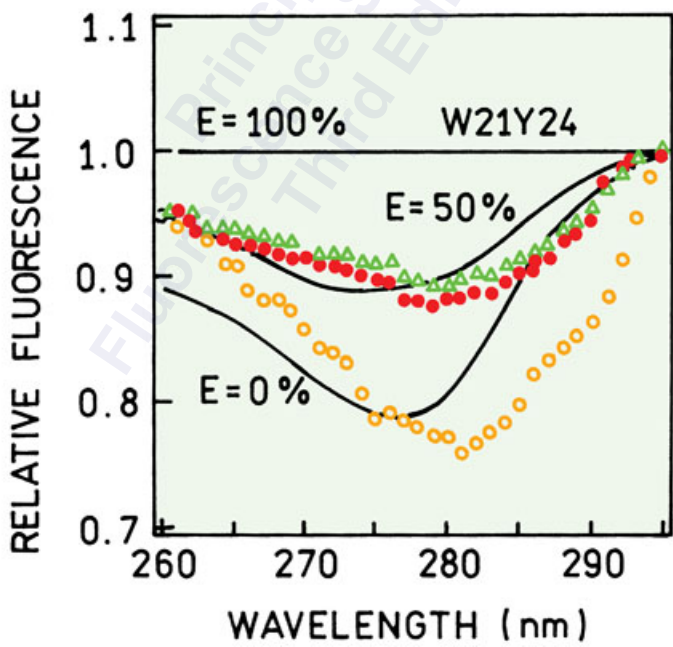
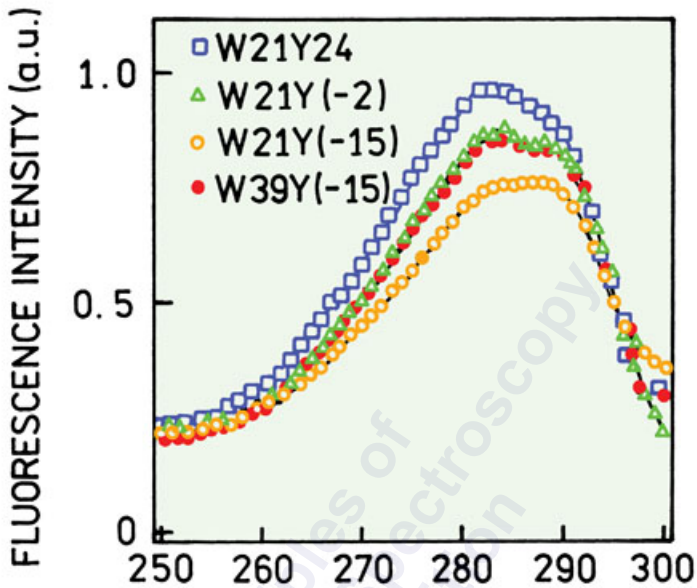
Head group region

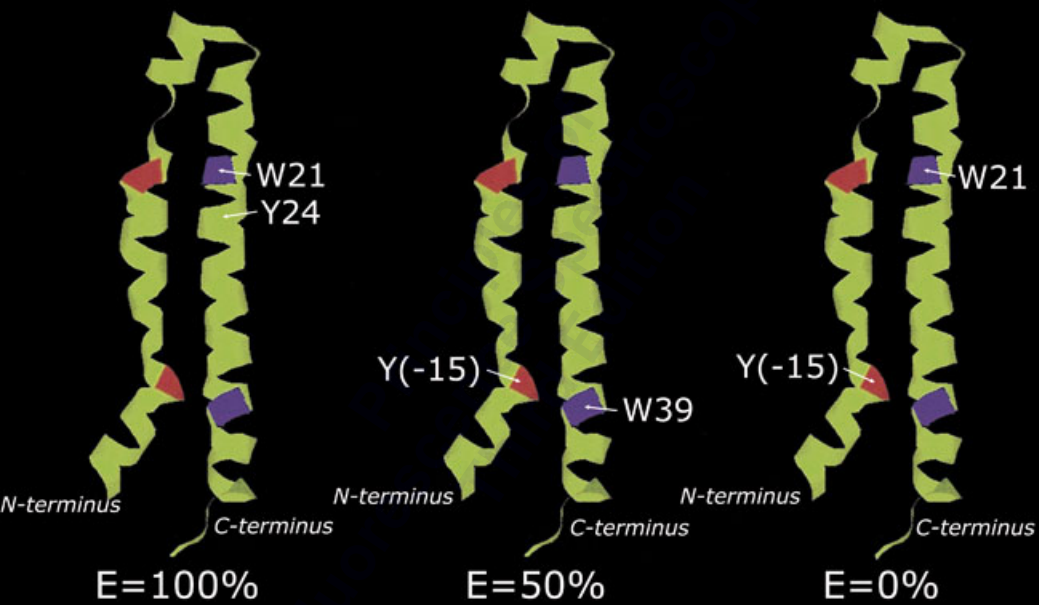
Hydrophobic core

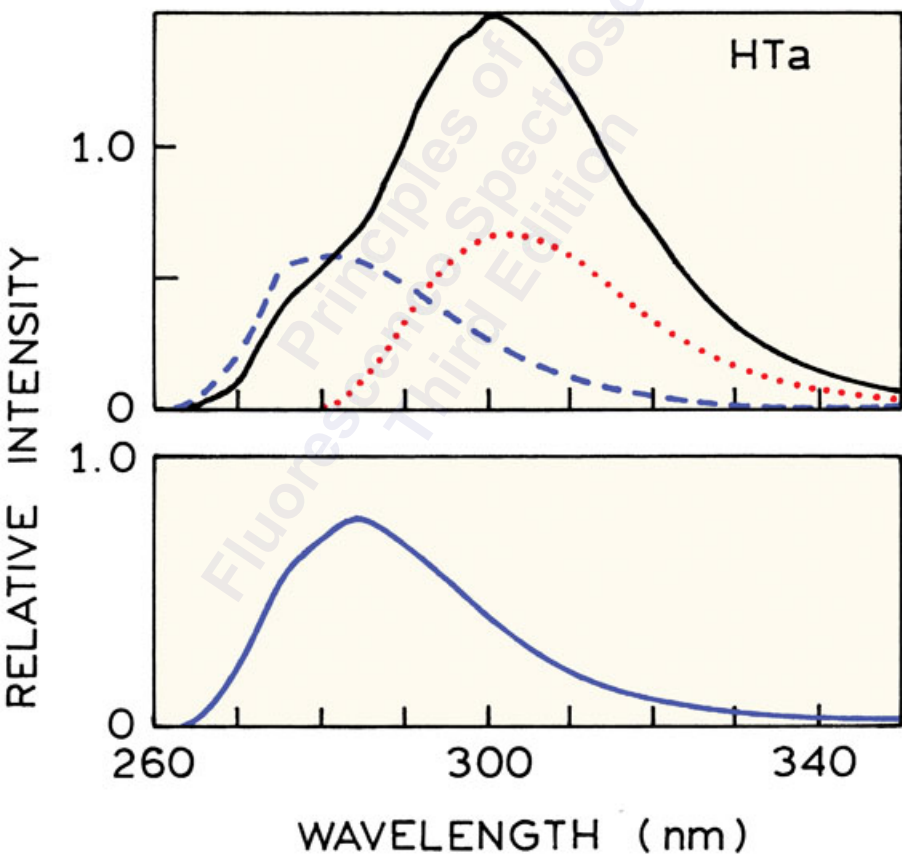
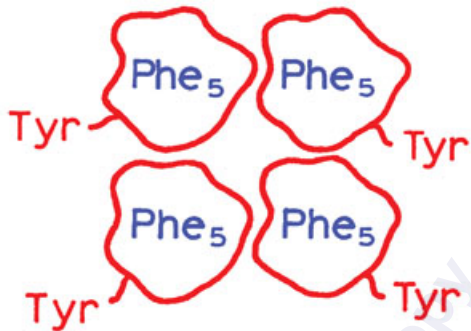


FLUORESCENCE INTENSITY (a.u.)











ABSORPTION

0.16  
0.08  
0

Phe + Tyr

Tyr

HTa

FLUORESCENCE

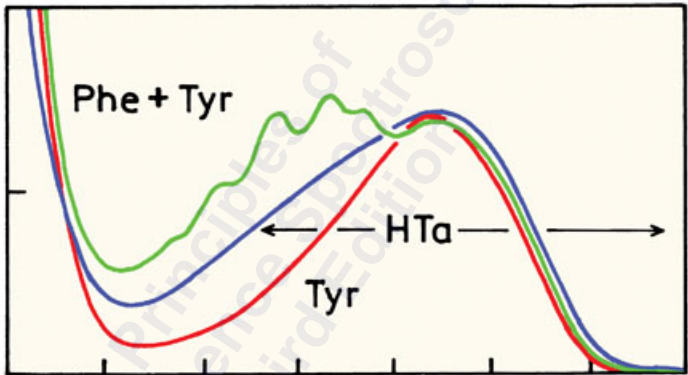
240

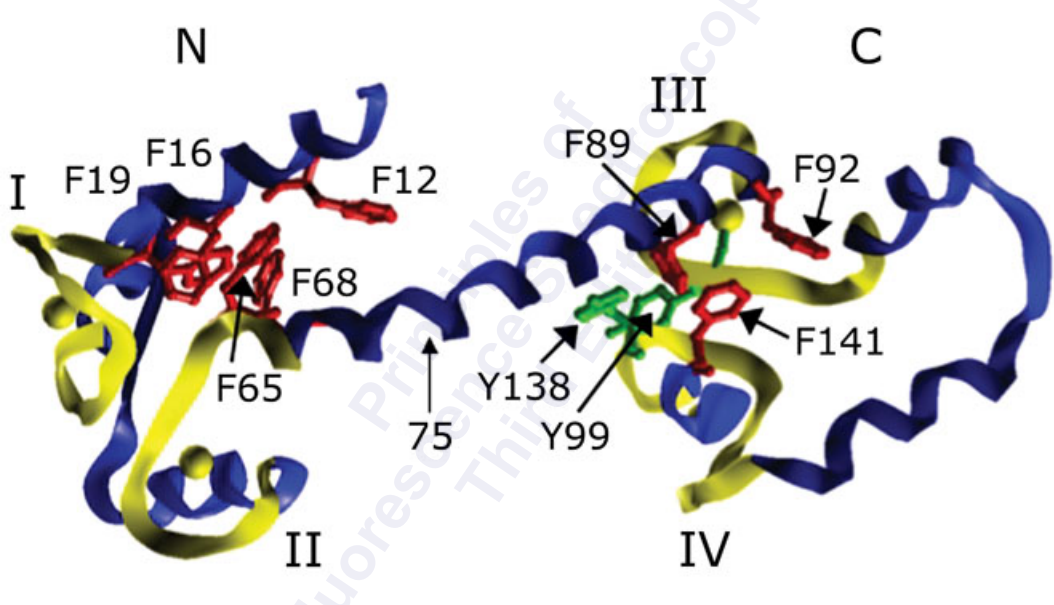
260

280

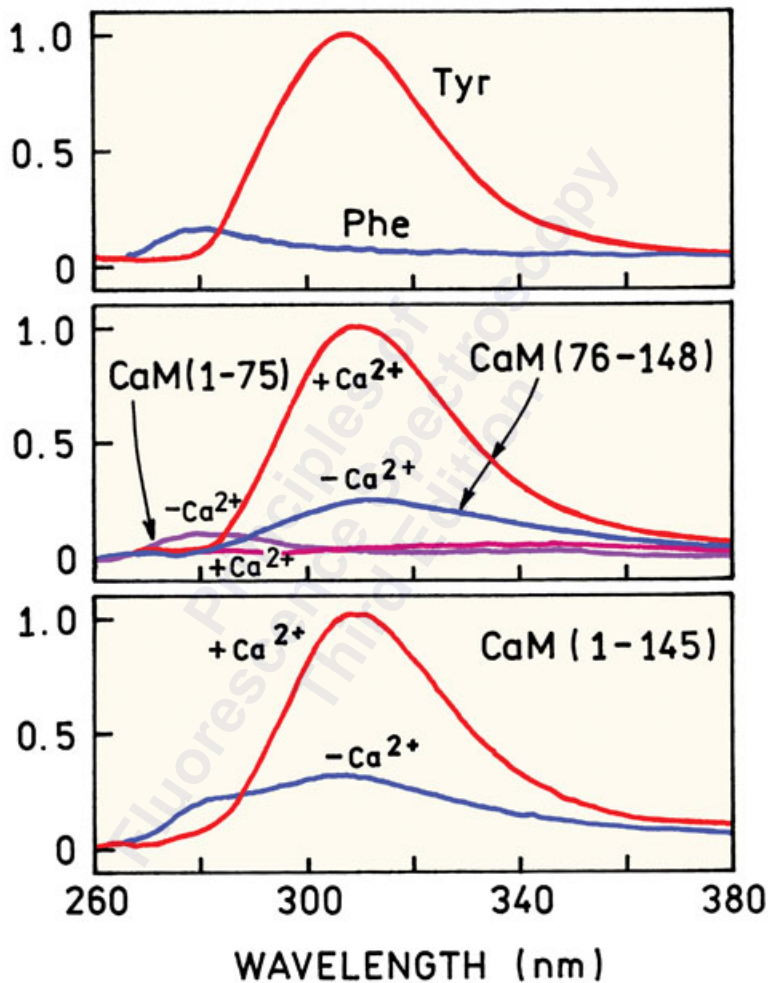
300

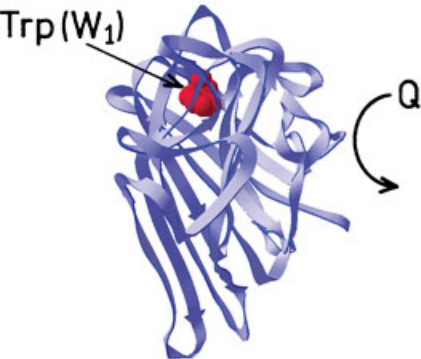
WAVELENGTH (nm)



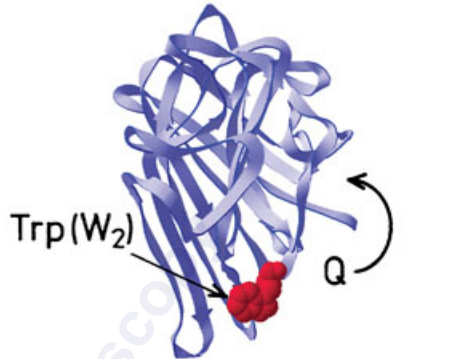


NORMALIZED FLUORESCENCE INTENSITY

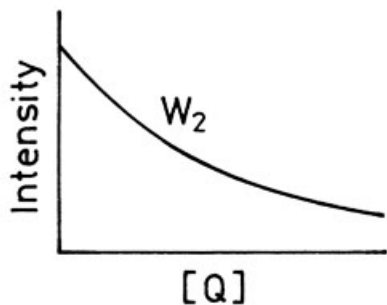
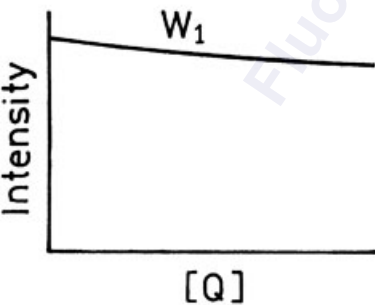
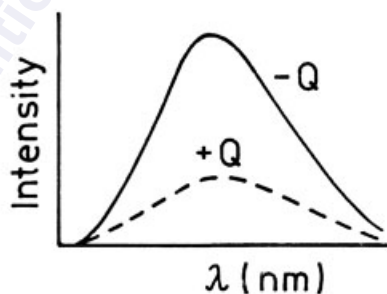


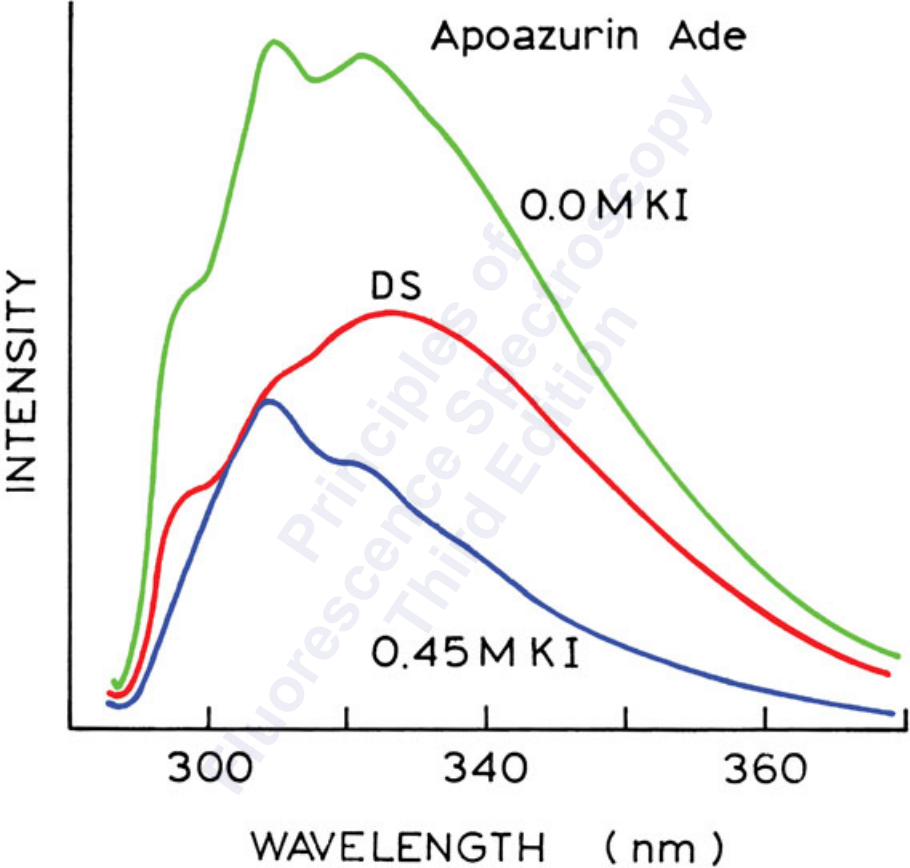


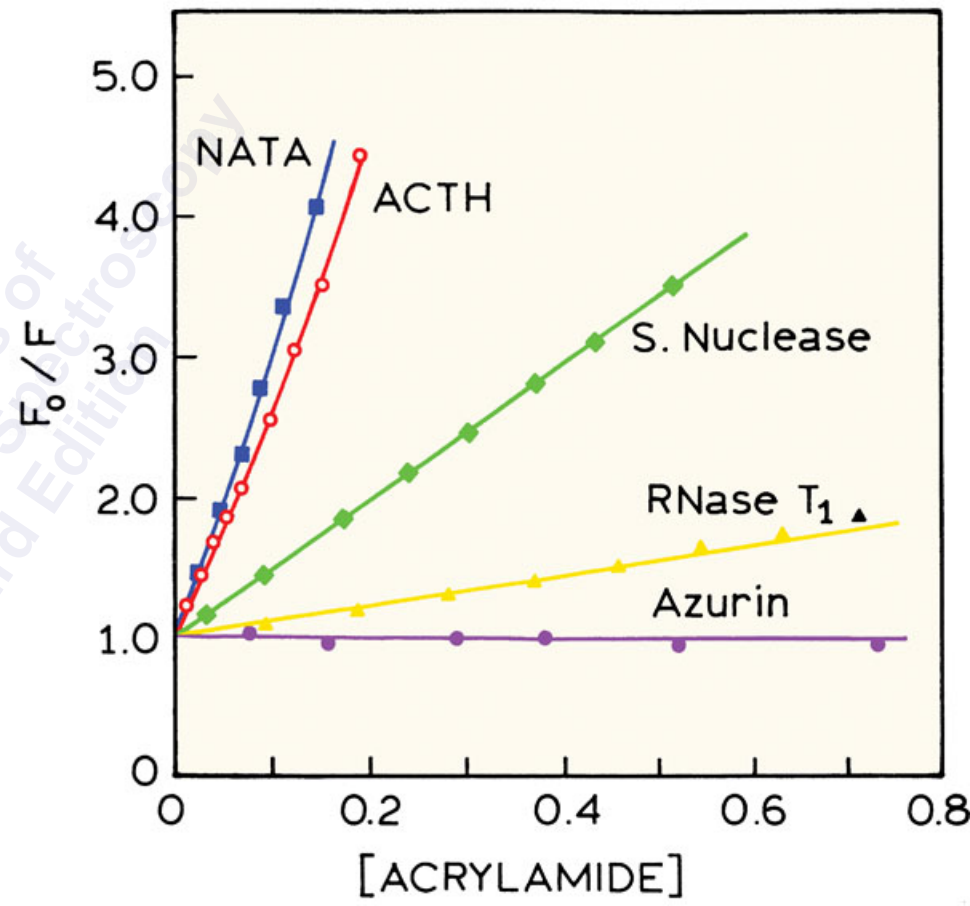
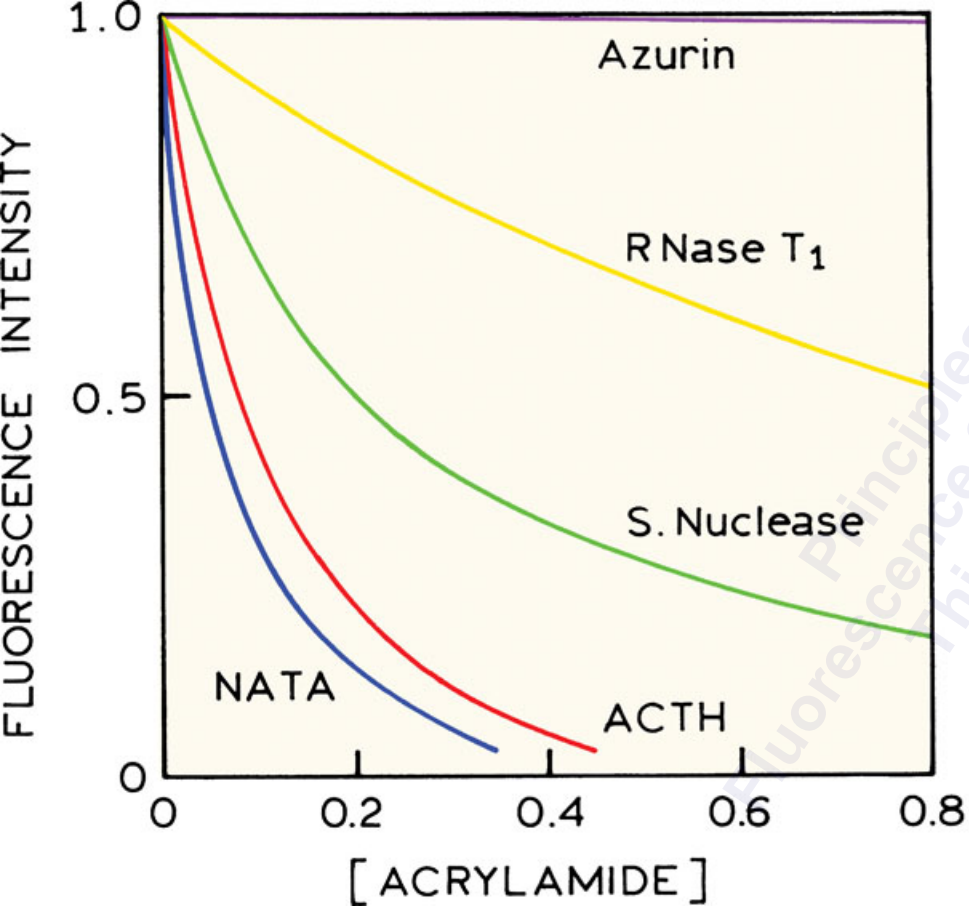
Buried Tryptophan

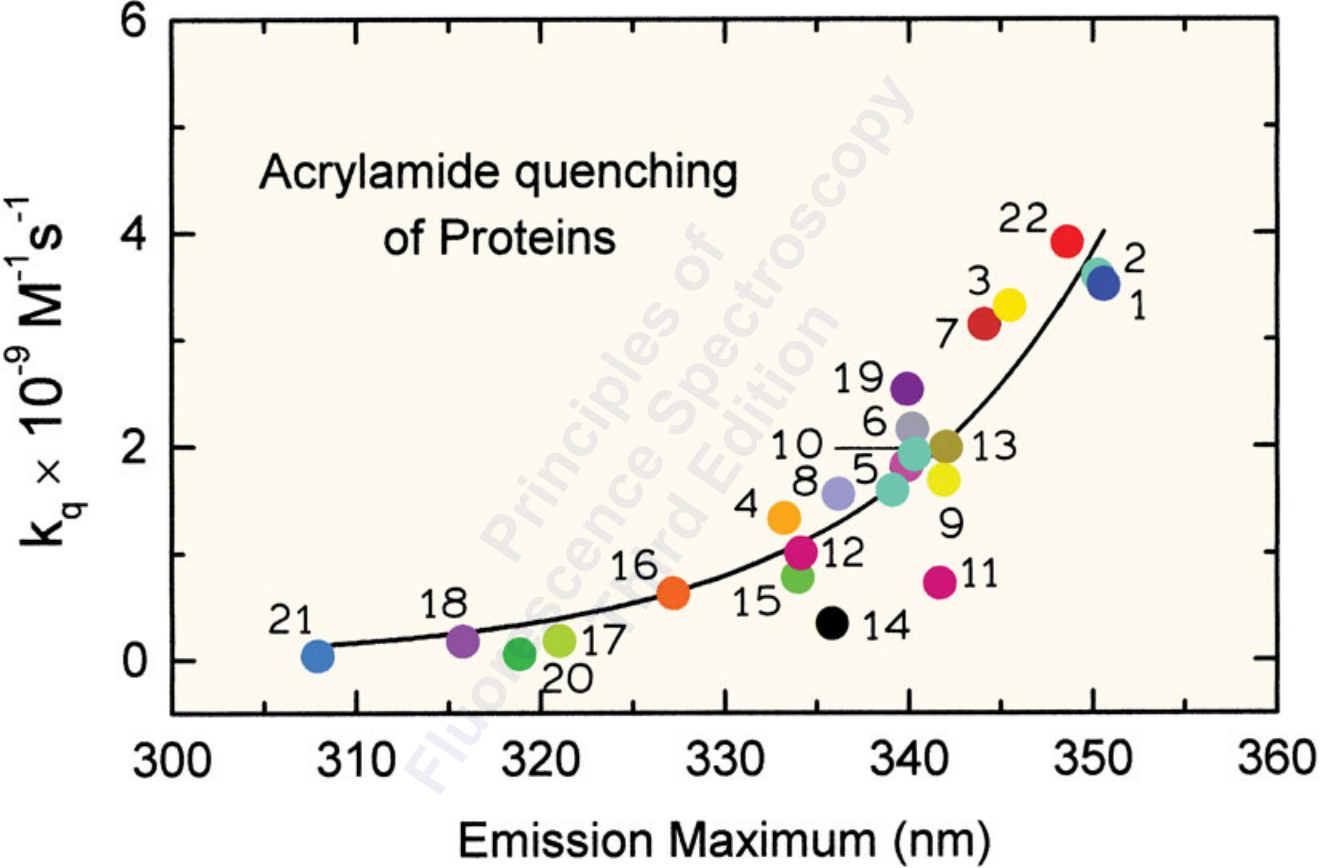


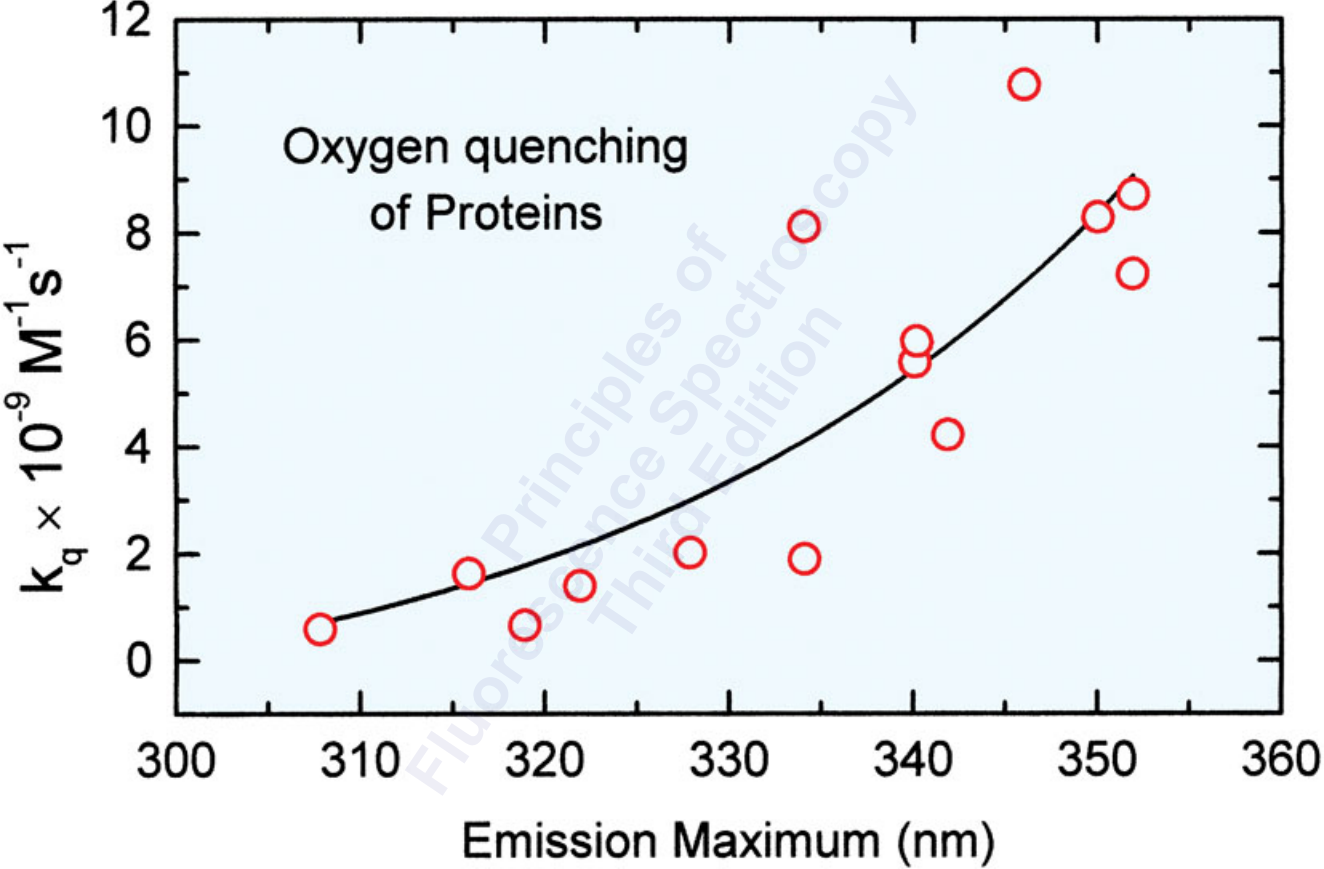
Surface Tryptophan



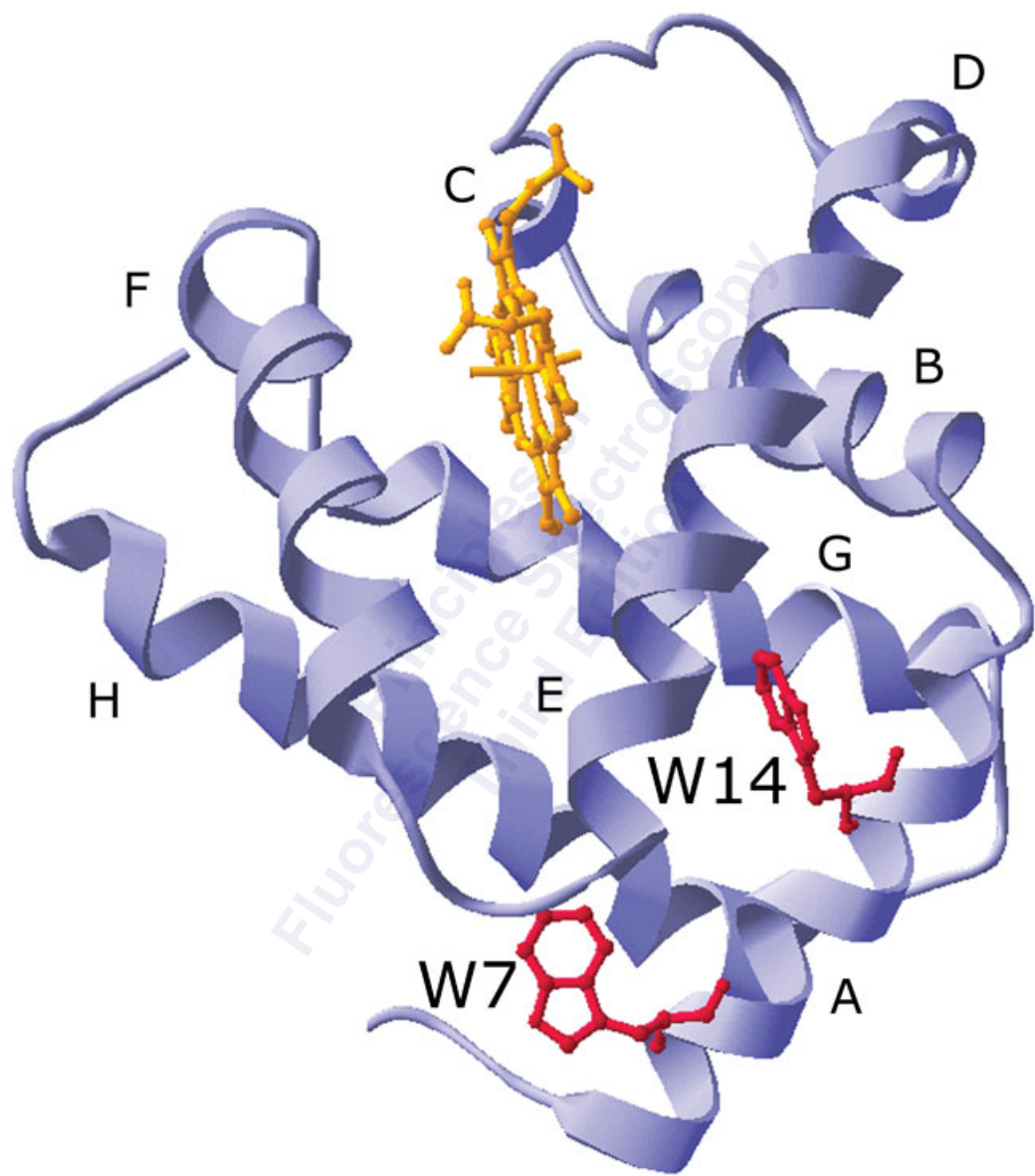


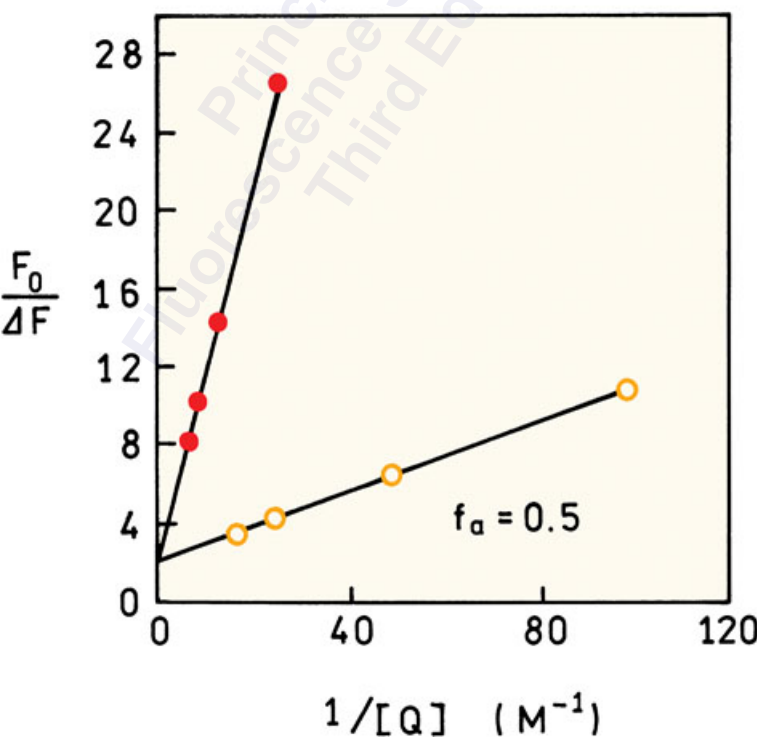
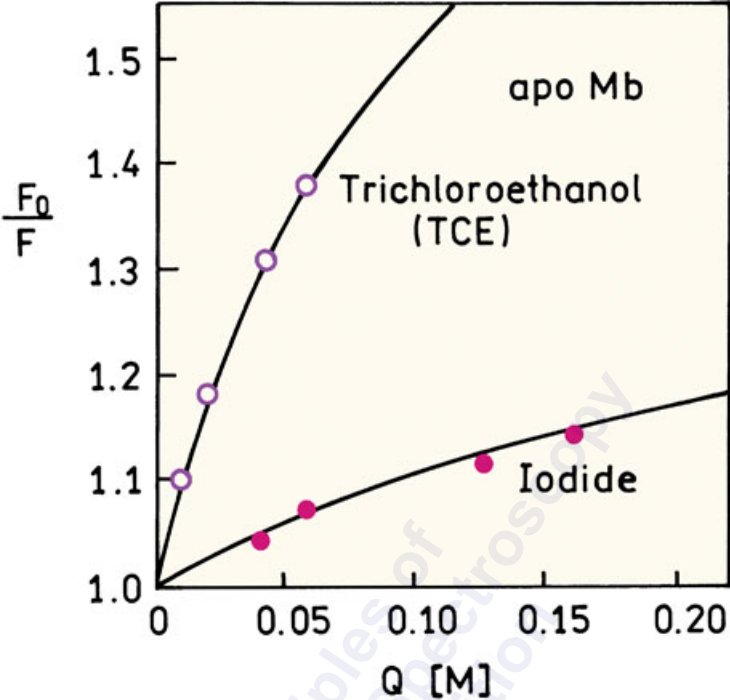


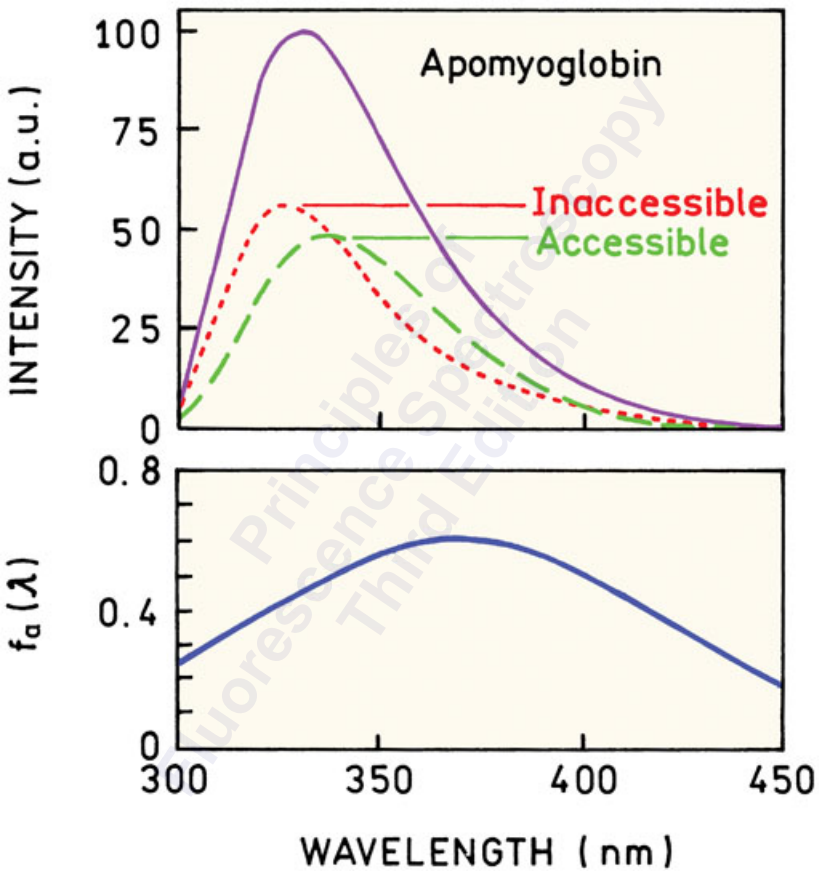


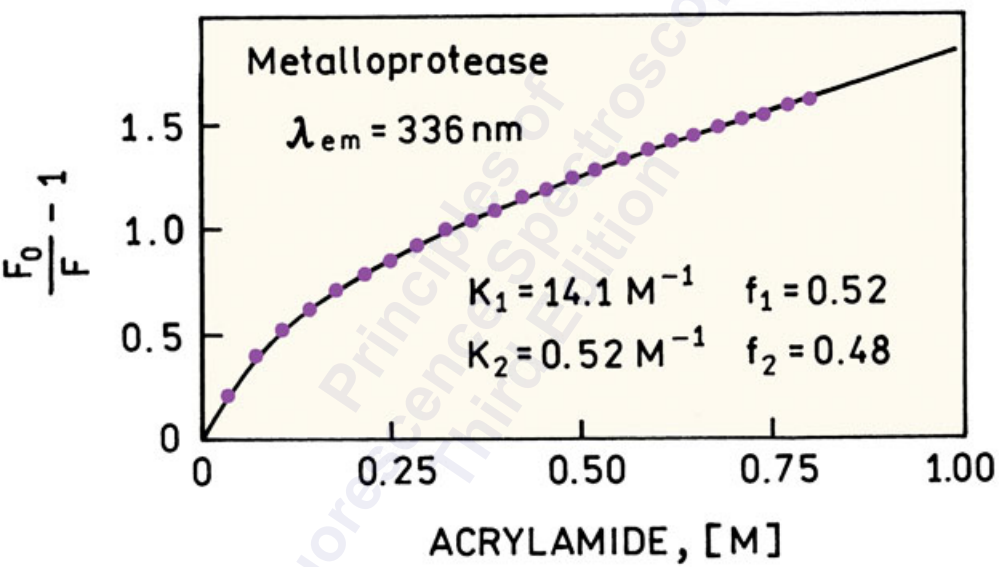


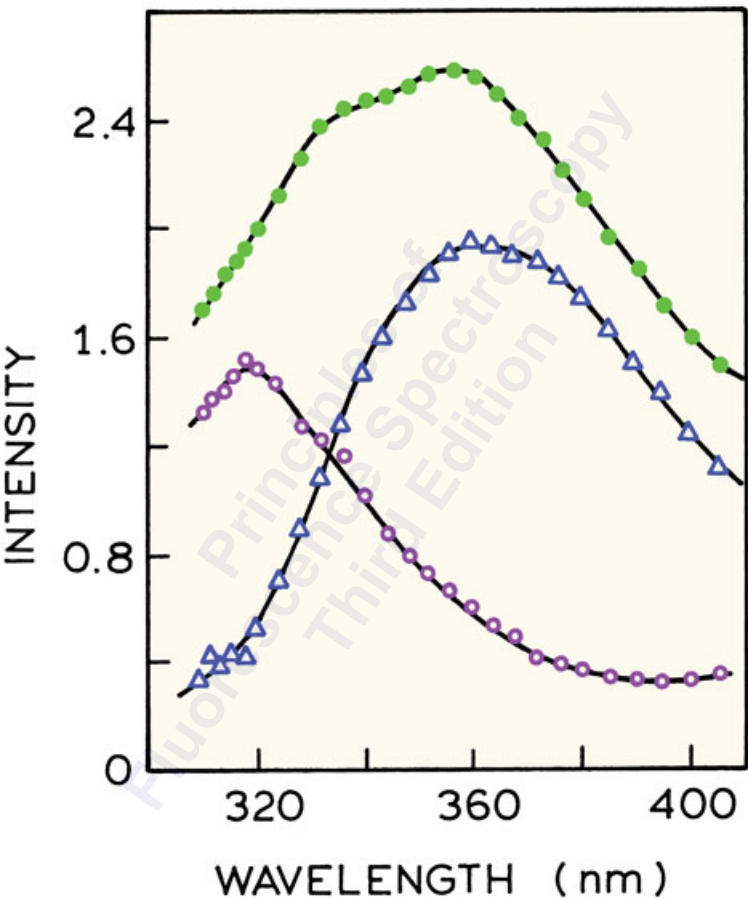




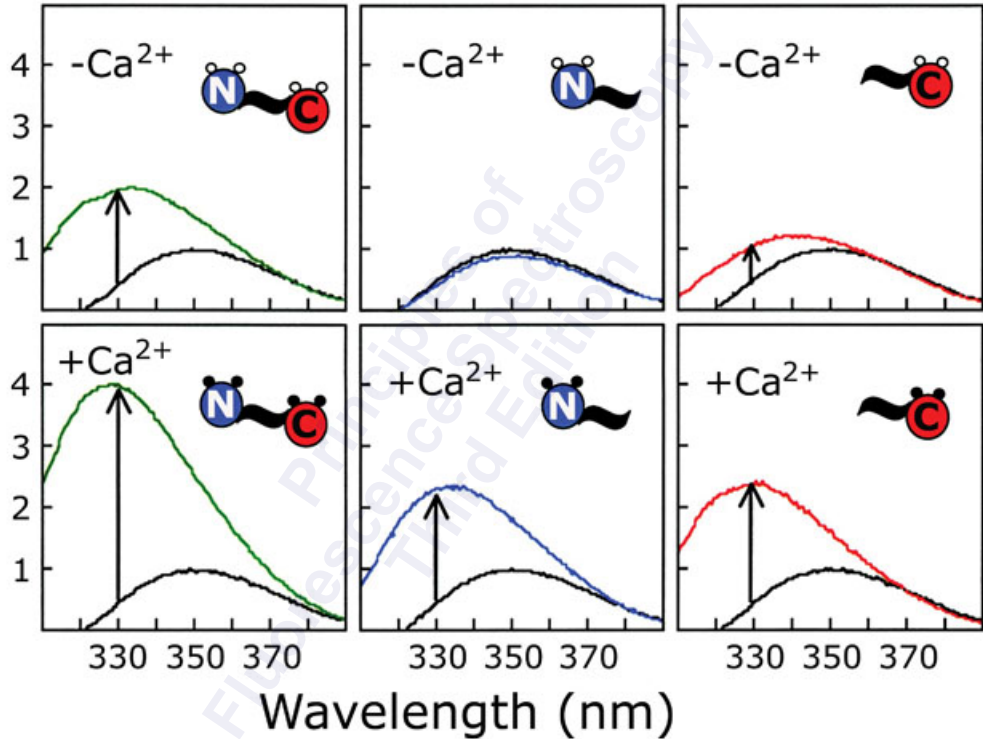


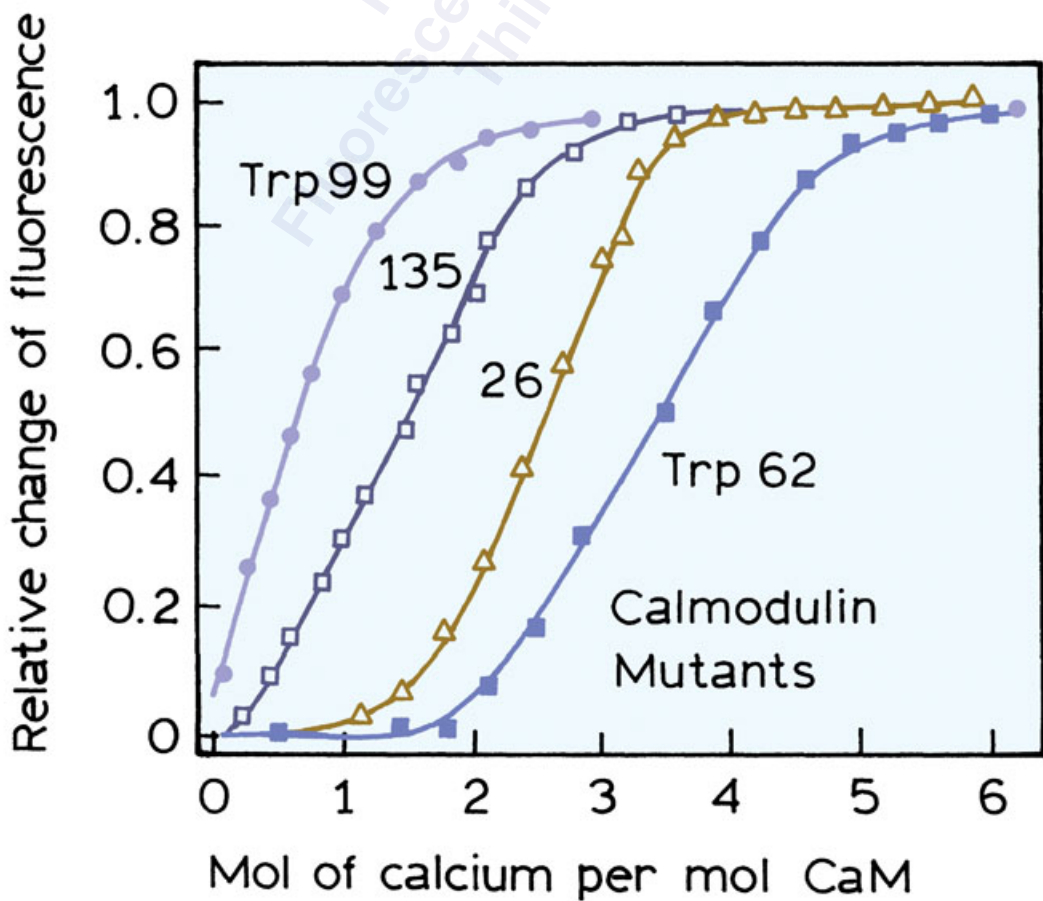
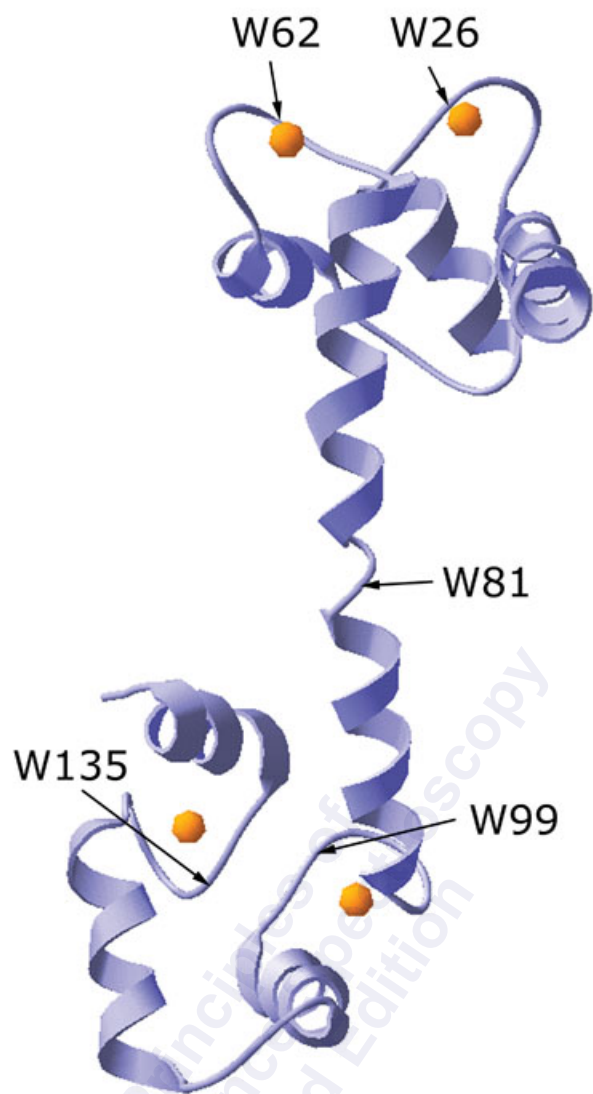


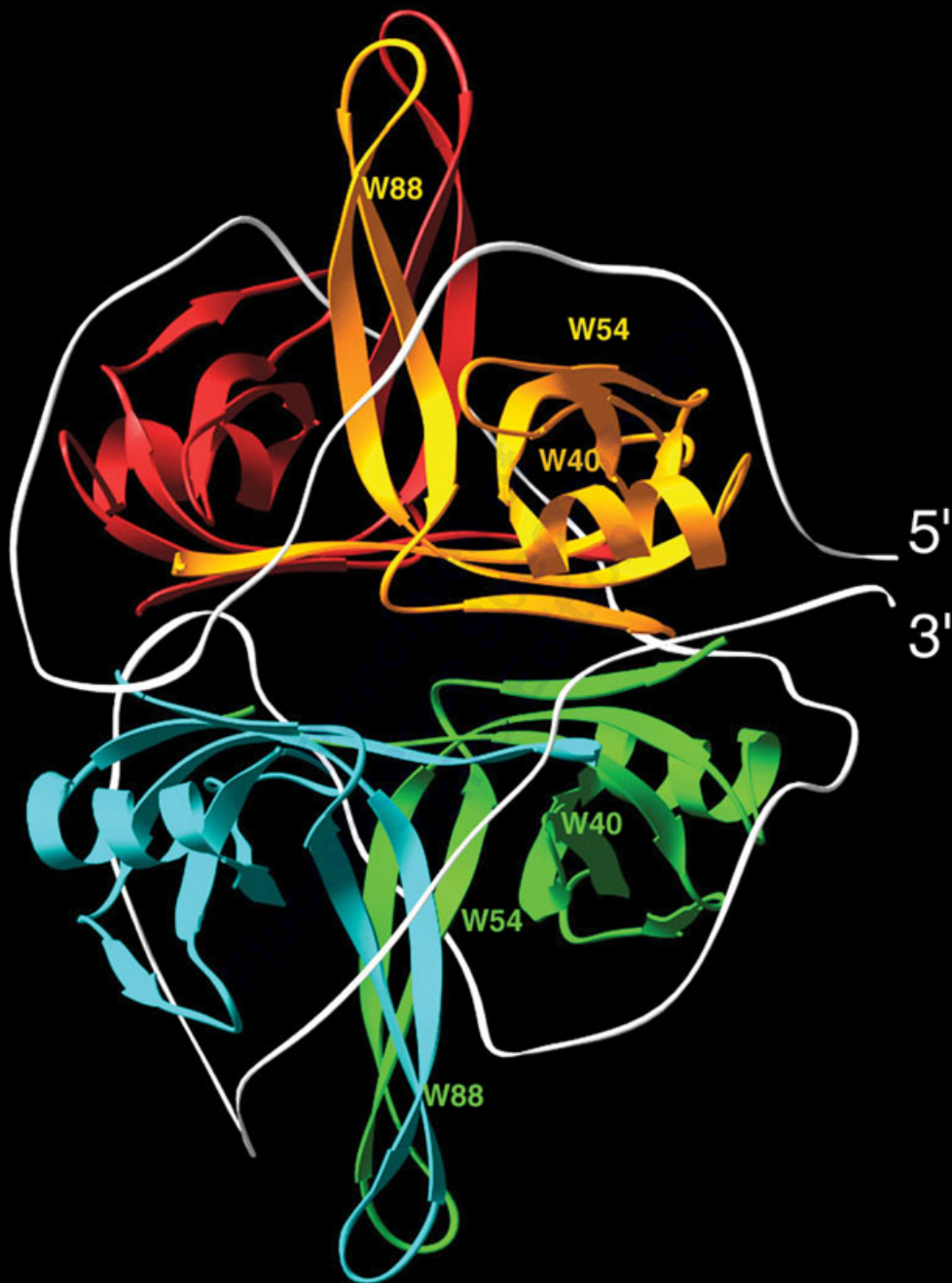




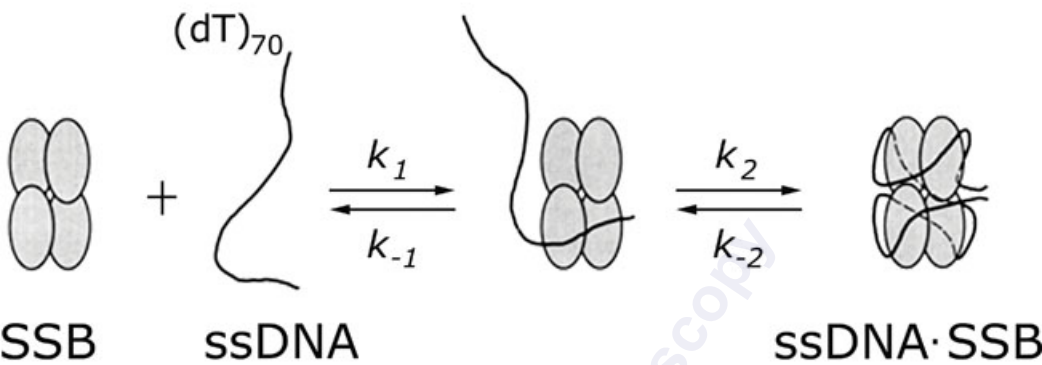
Normalized Fluorescence



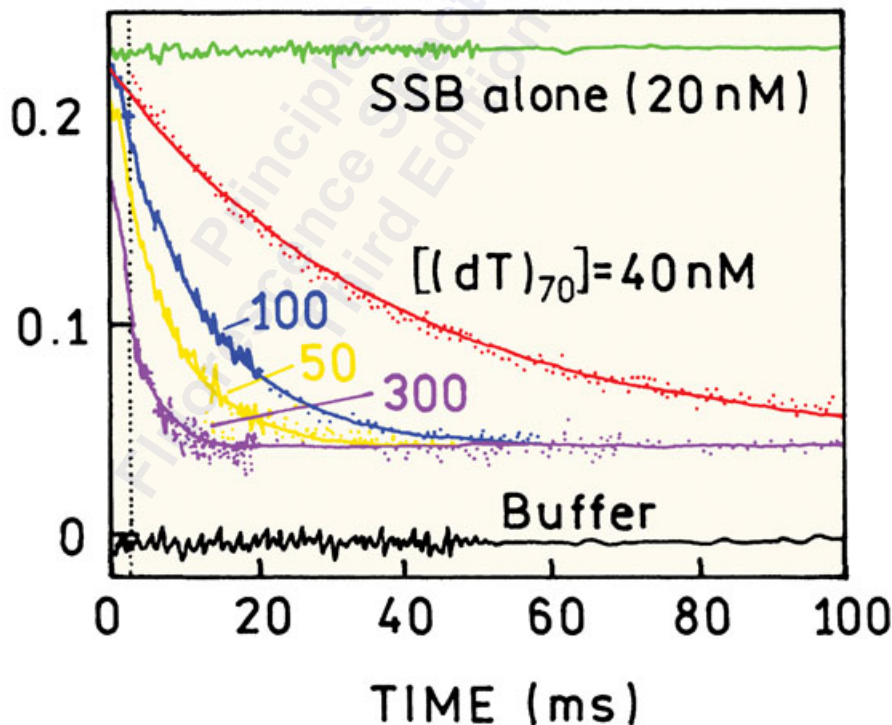


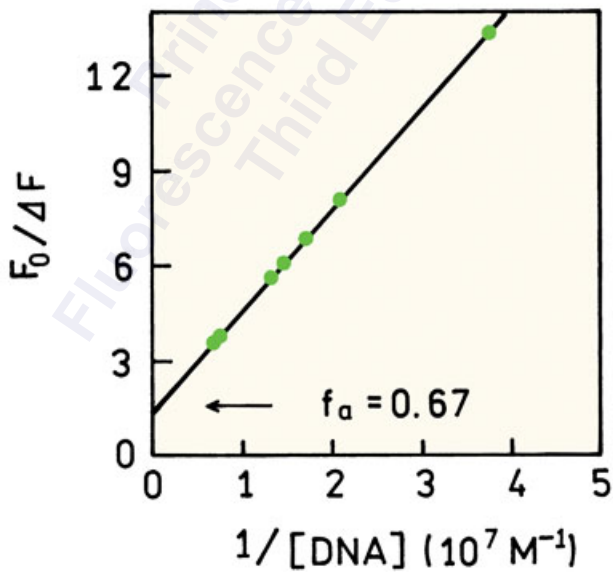
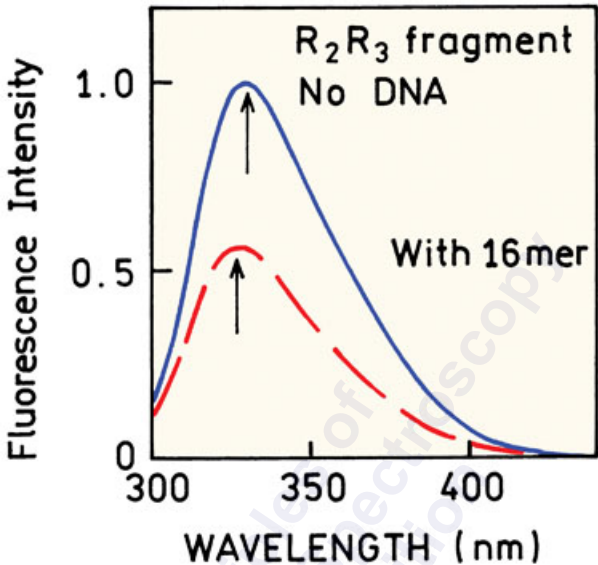


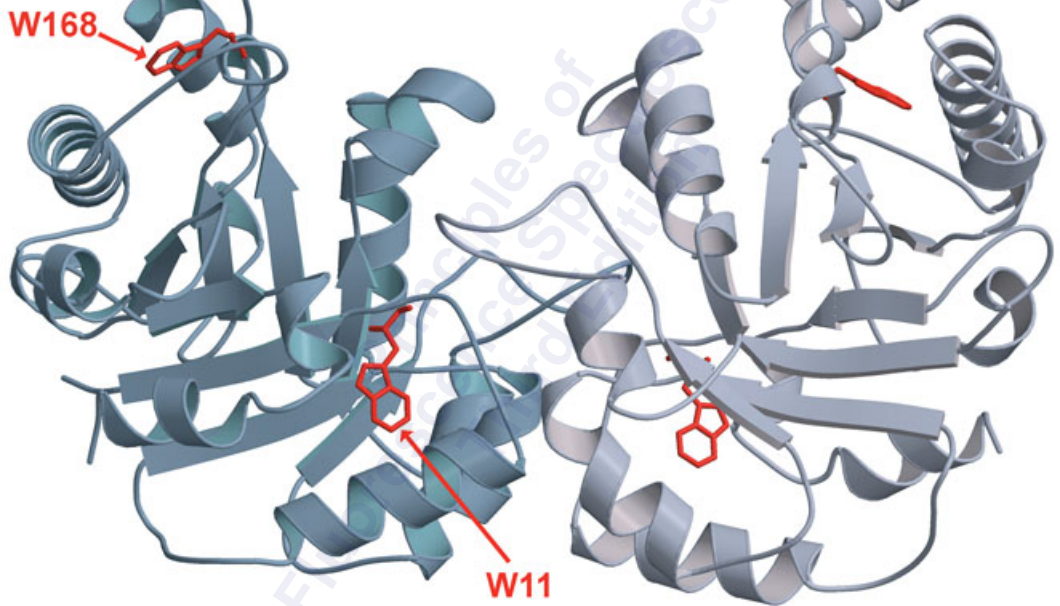




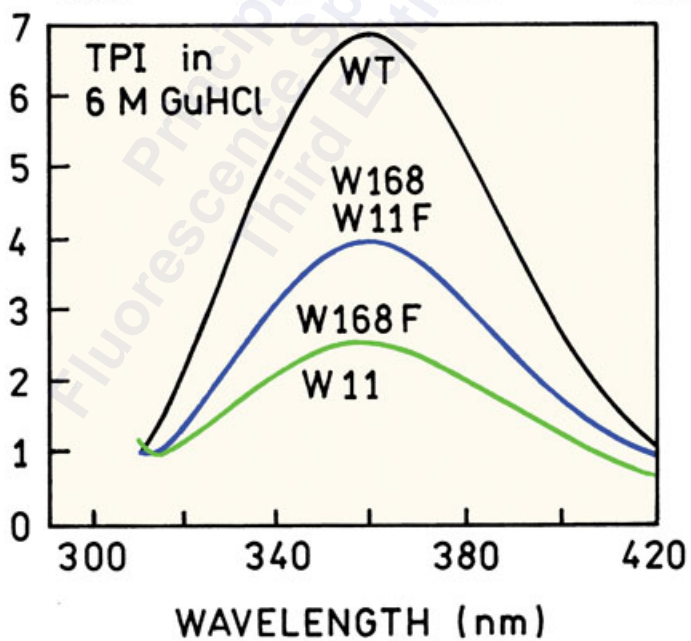
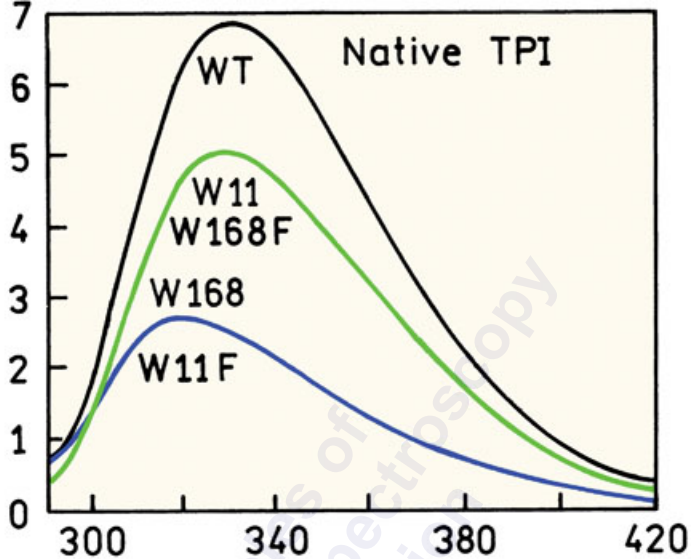
SSB Trp FLUORESCENCE

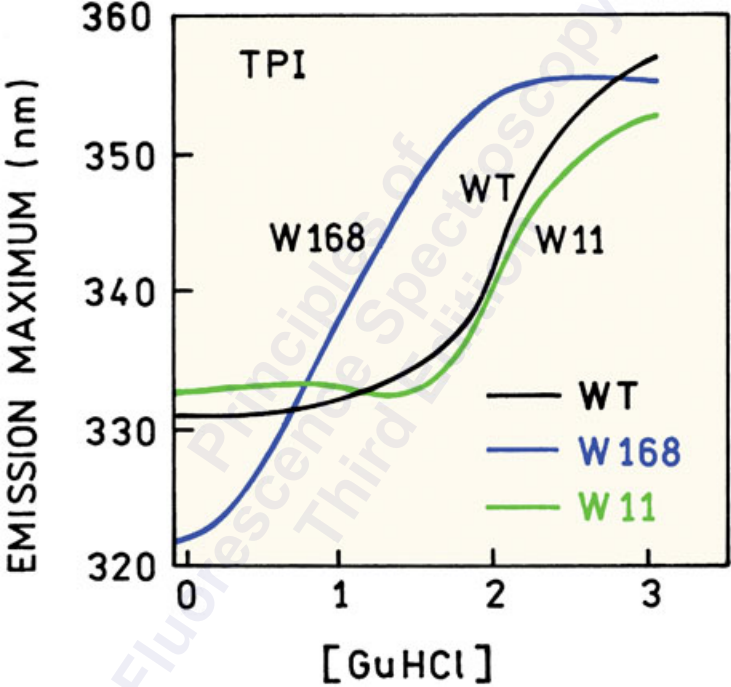


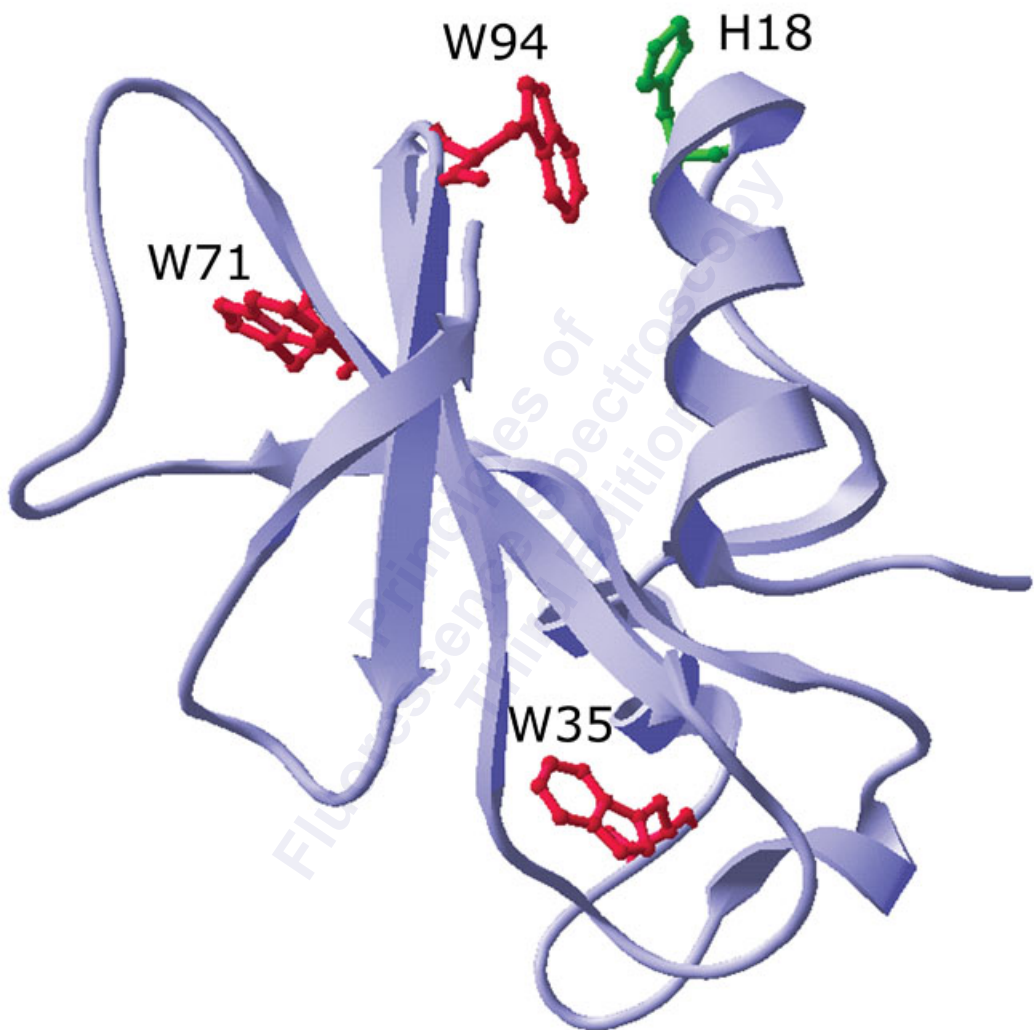


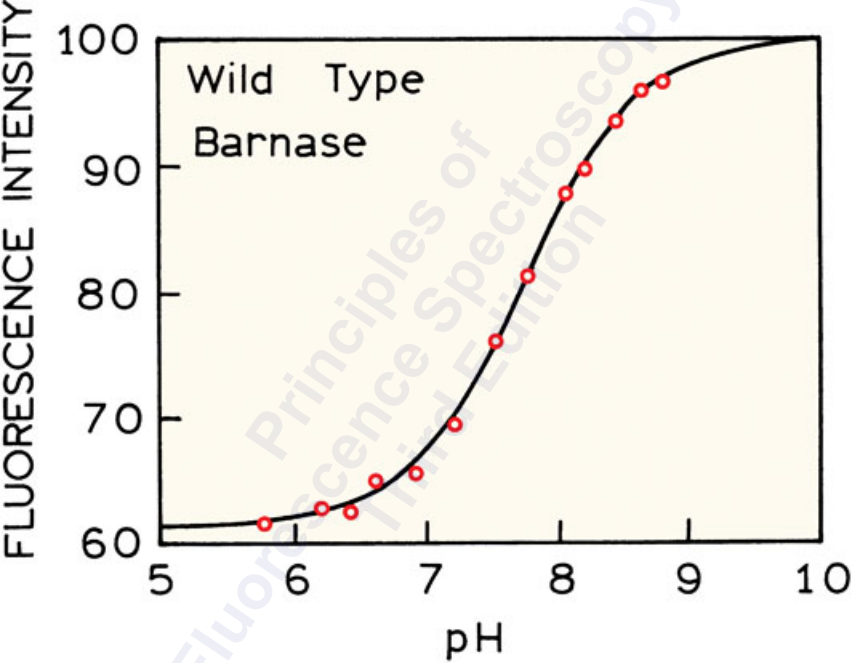


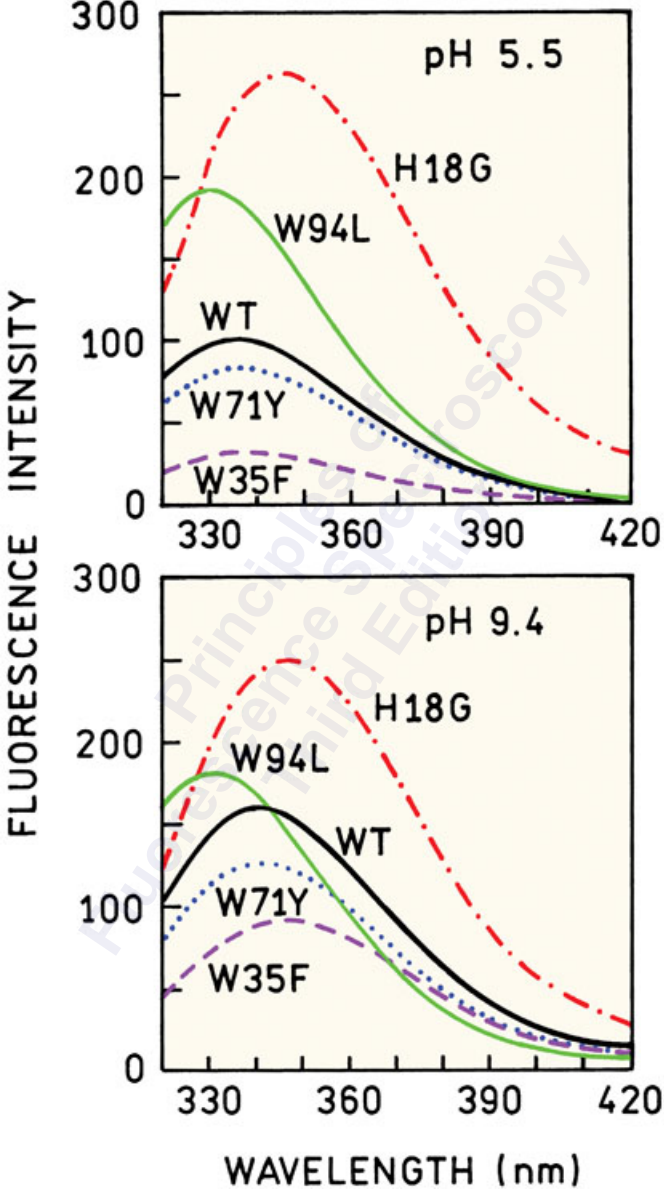
FLUORESCENCE INTENSITY (a.u.)







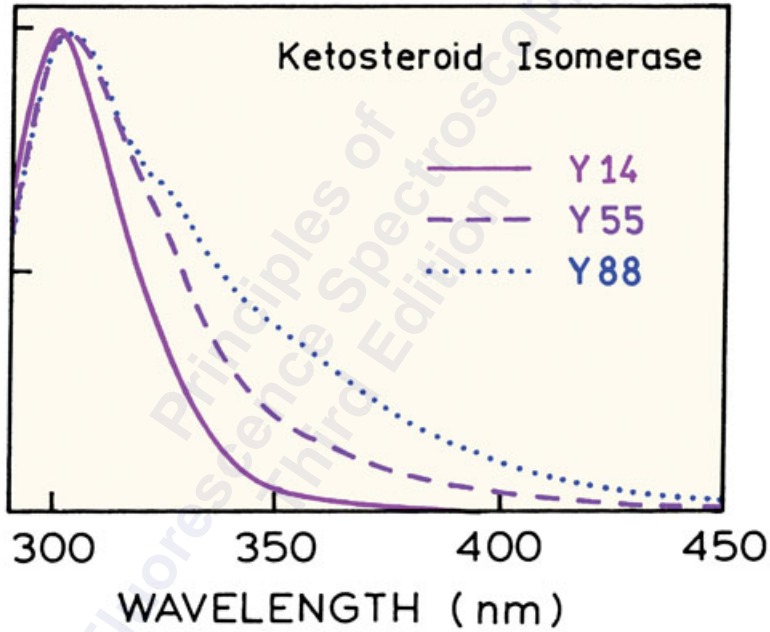




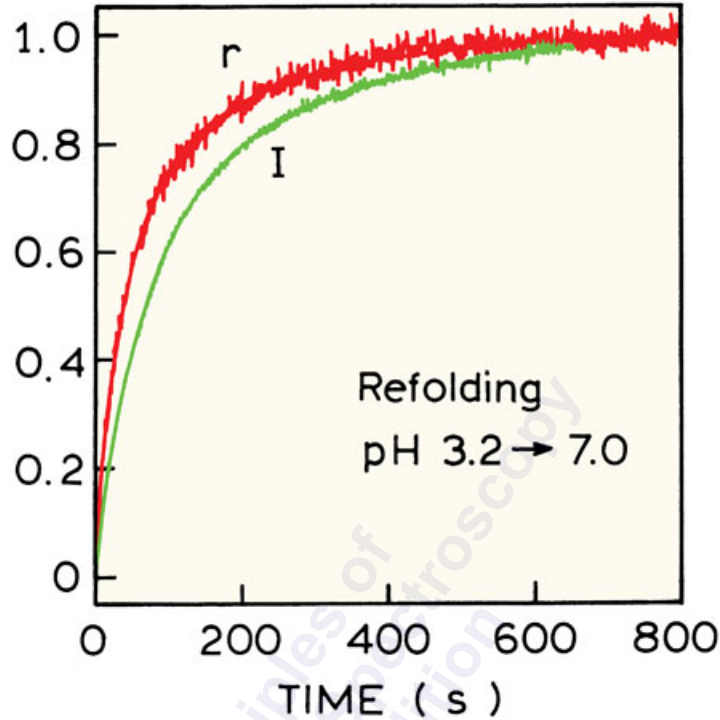


FLUORESCENCE  
INTENSITY

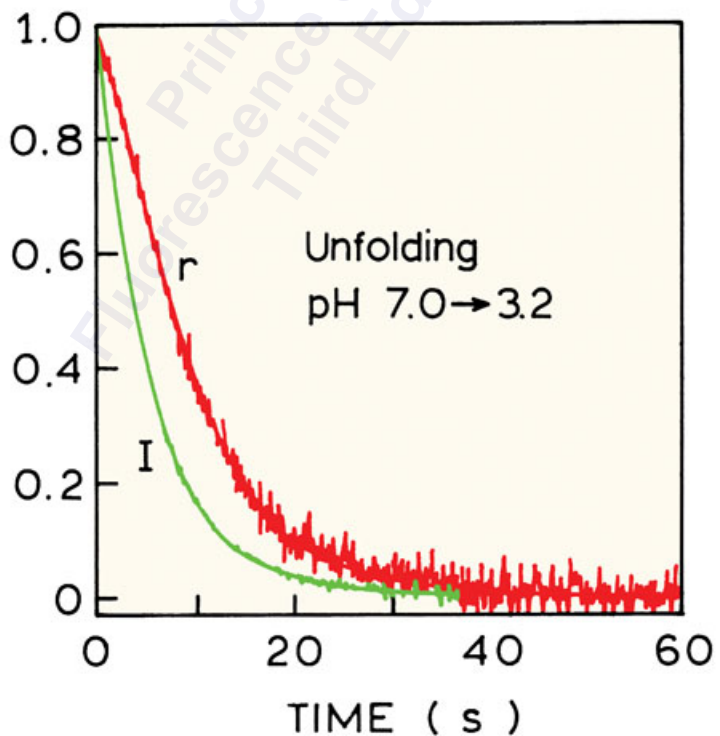
# Ketosteroid Isomerase

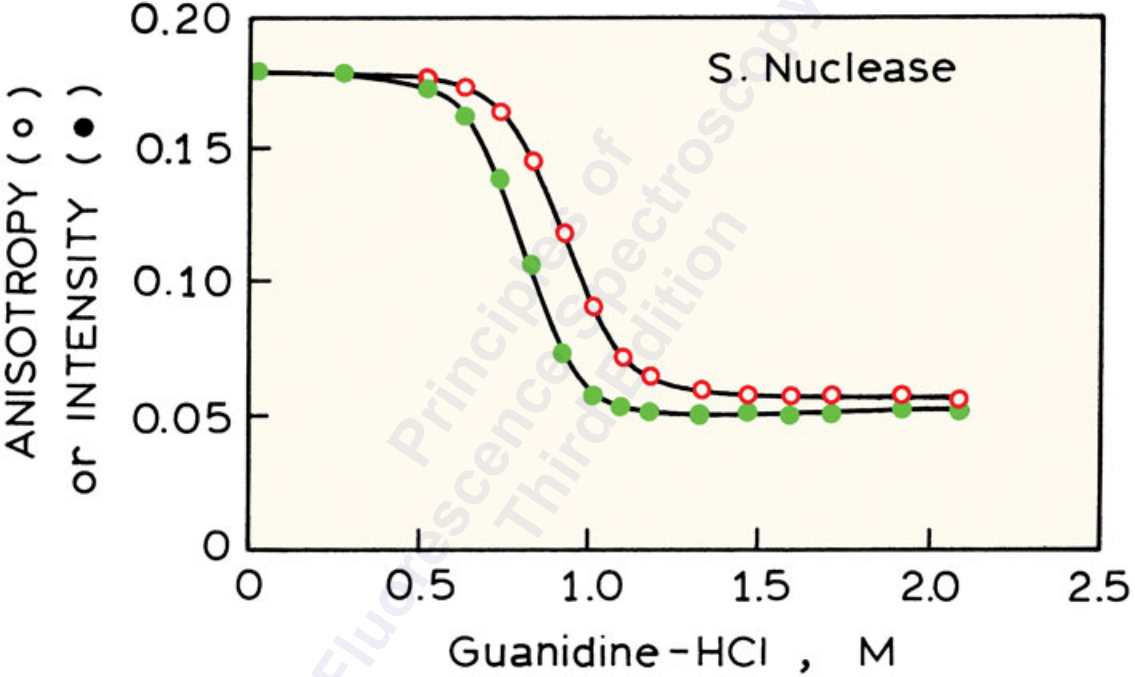


OBSERVABLE CHANGE



OBSERVABLE CHANGE





RELATIVE FLUORESCENCE

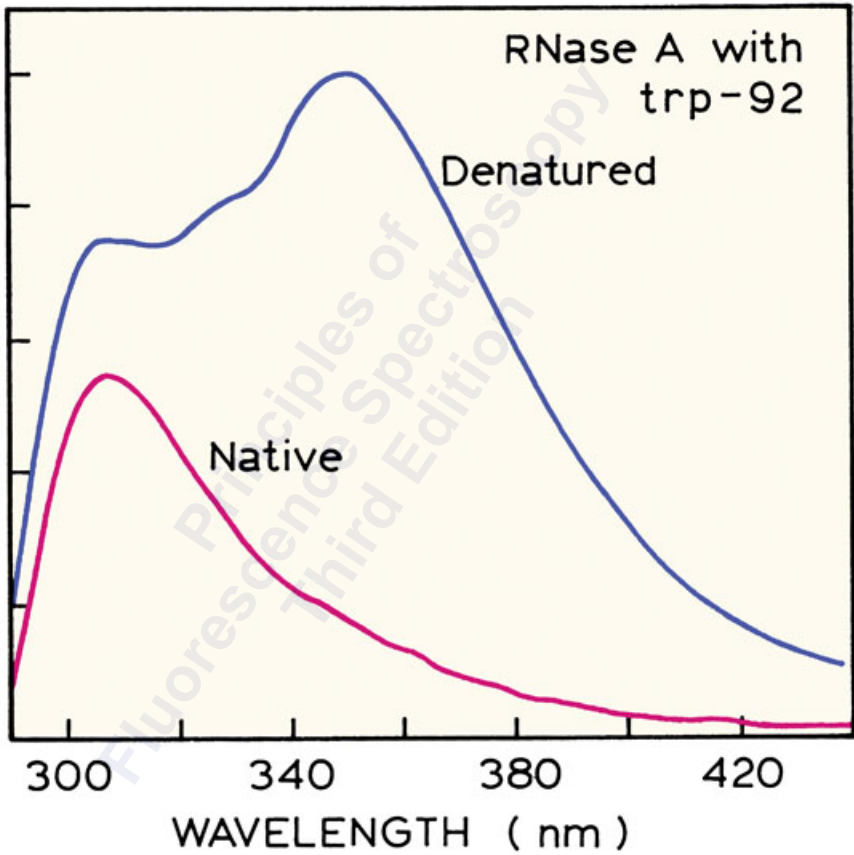
1.0  
0.8  
0.6  
0.4  
0.2  
0

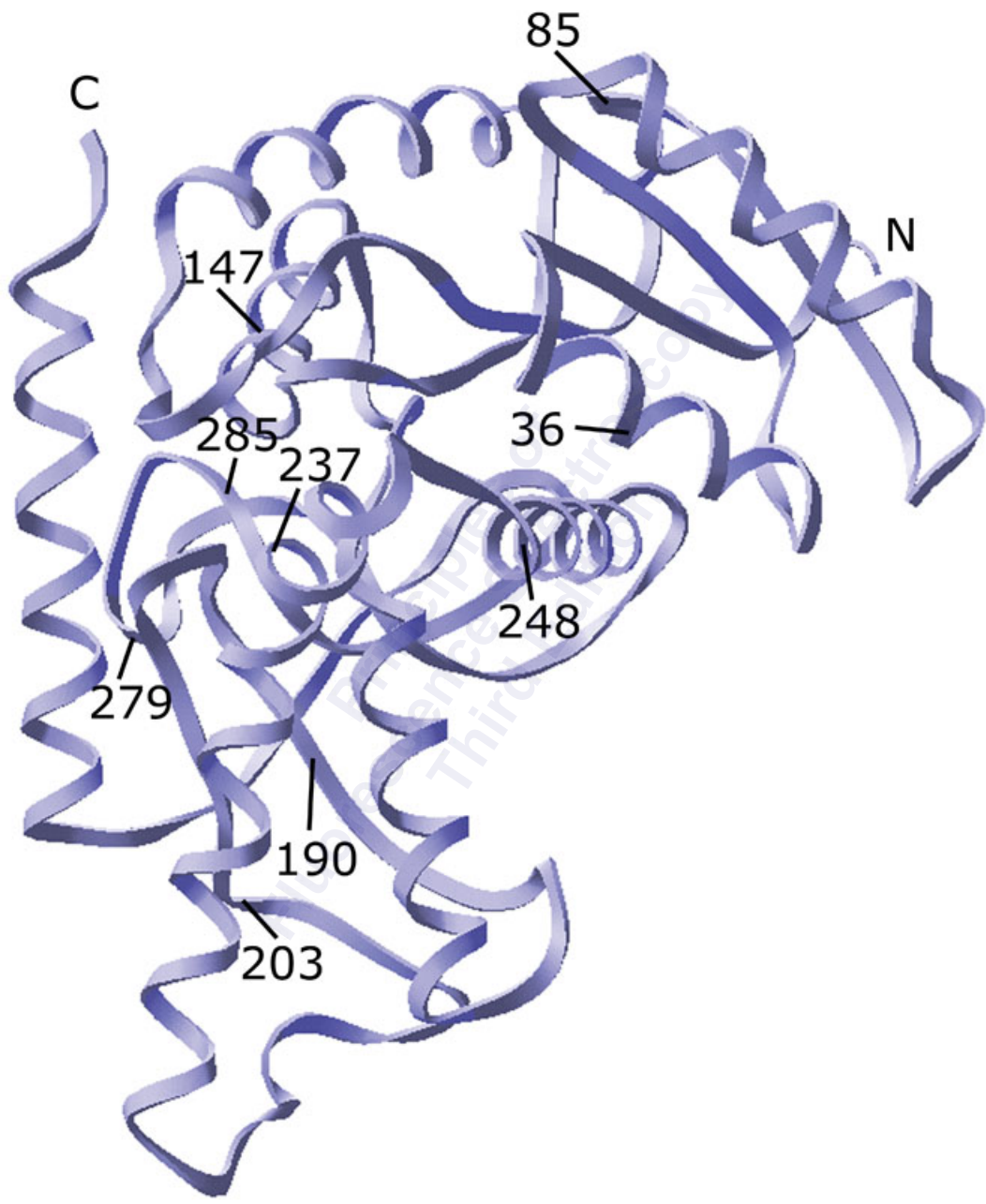
RNase A with  
trp-92

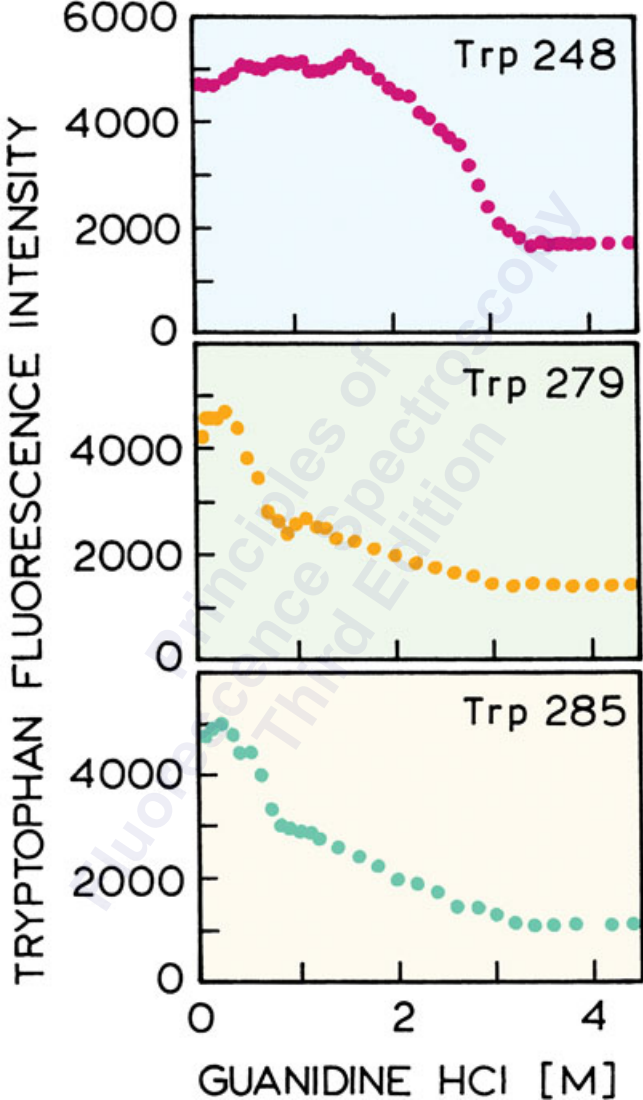
Denatured

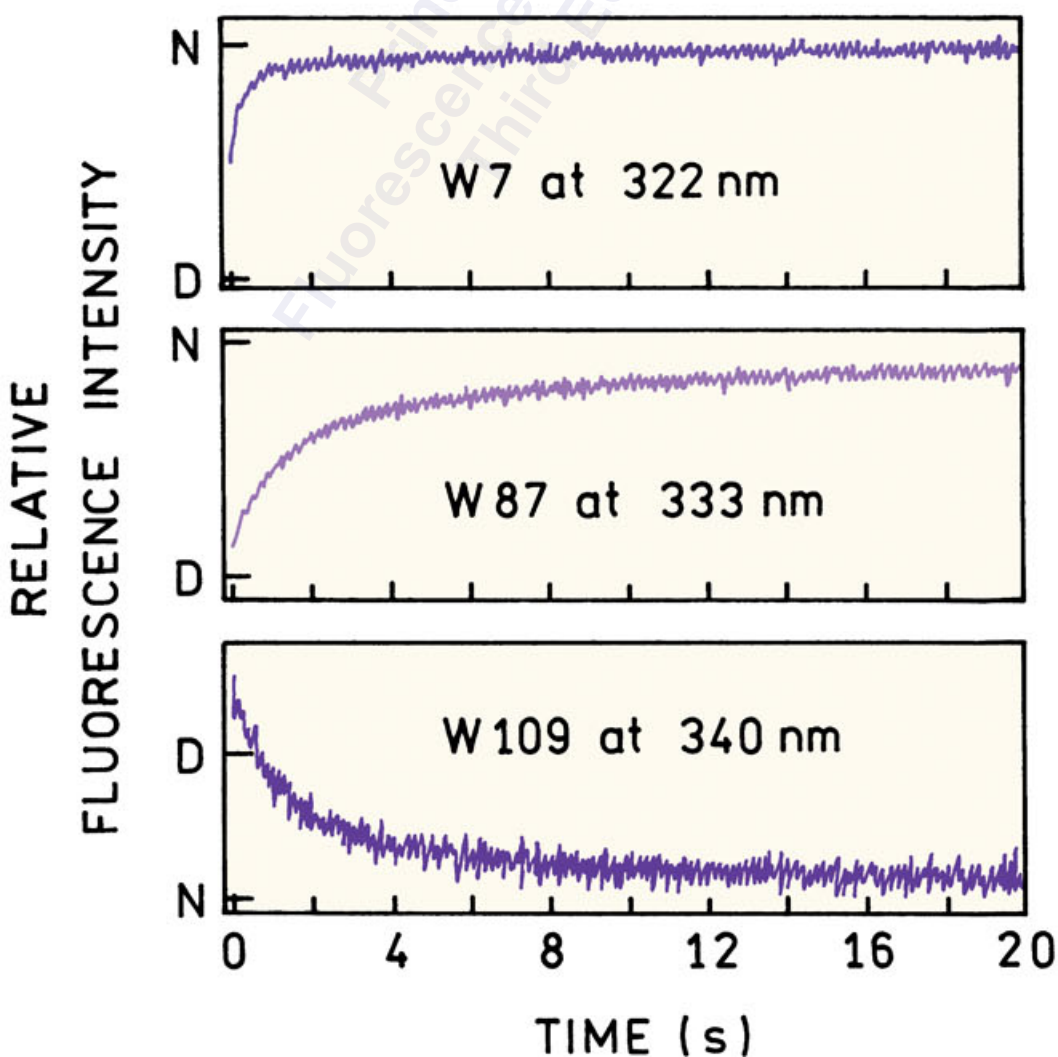
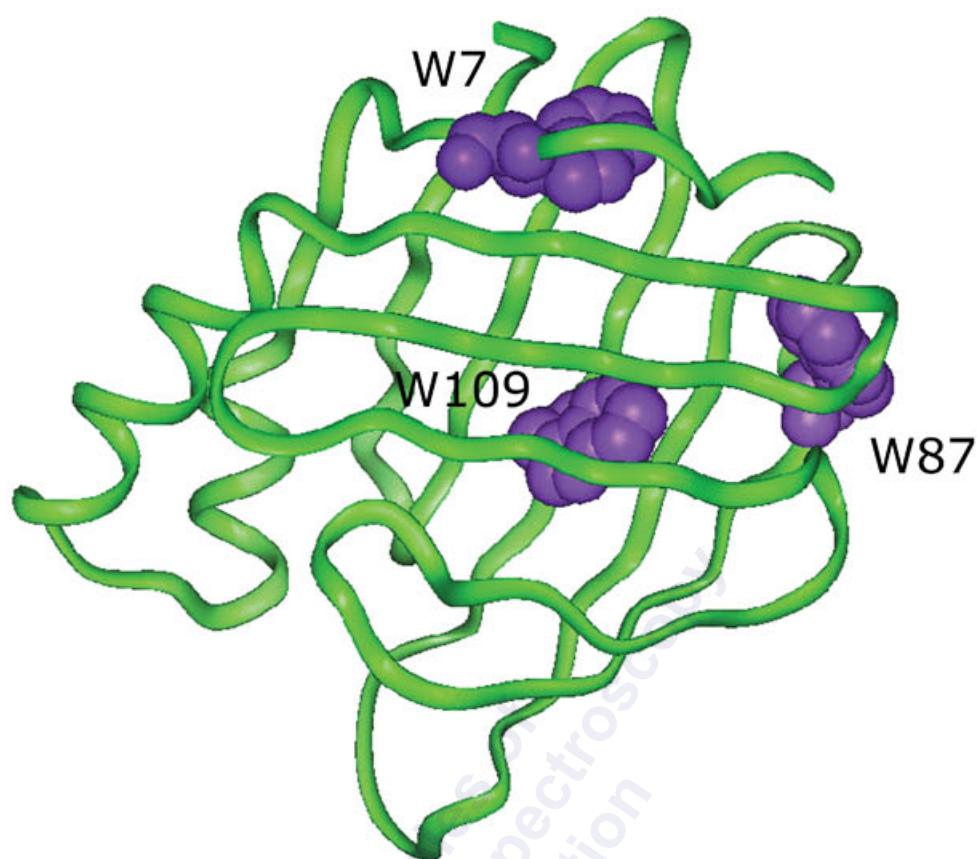
Native

WAVELENGTH ( nm )











Reactive Center  
Loop

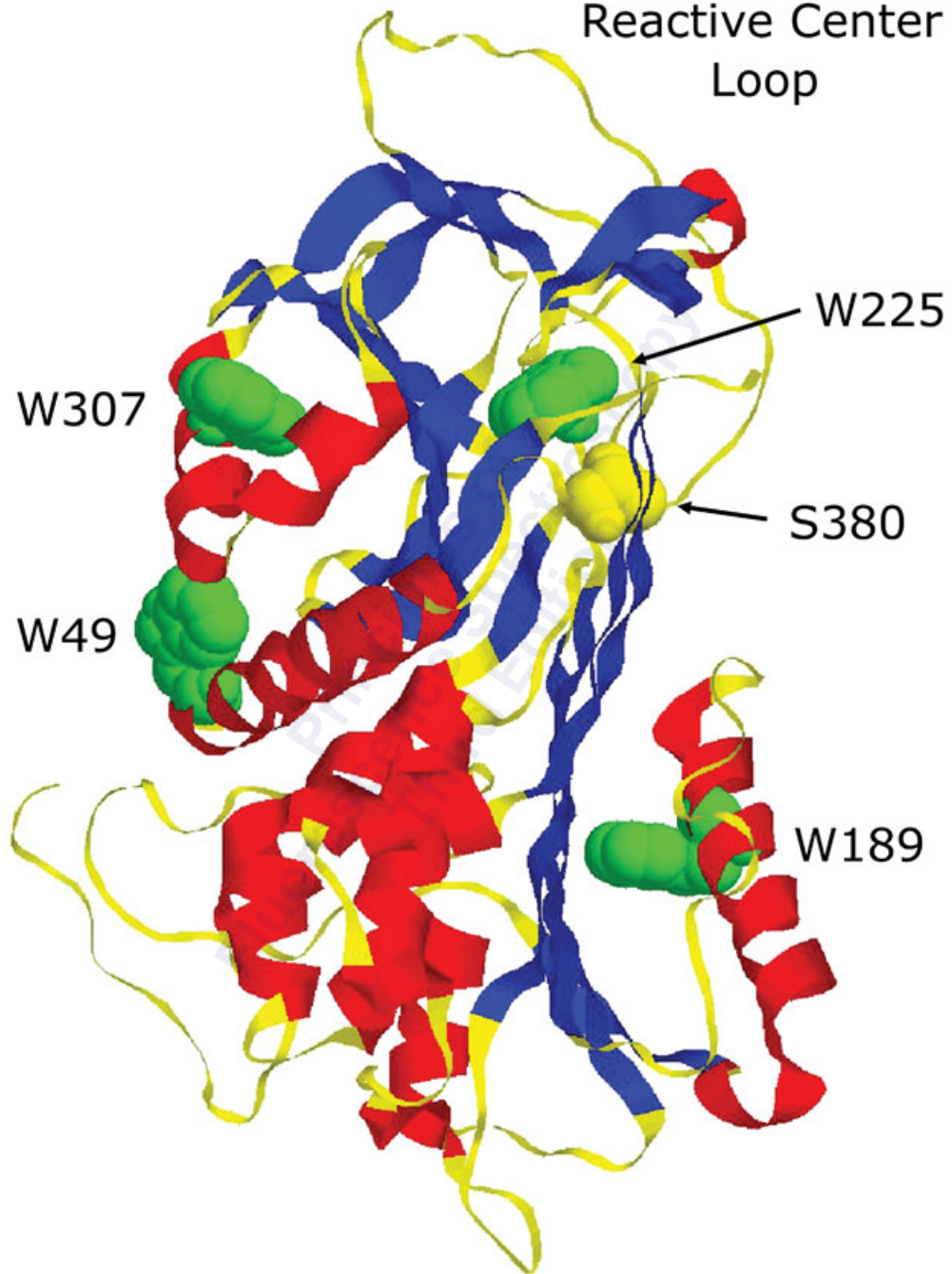
W225

S380

W307

W49

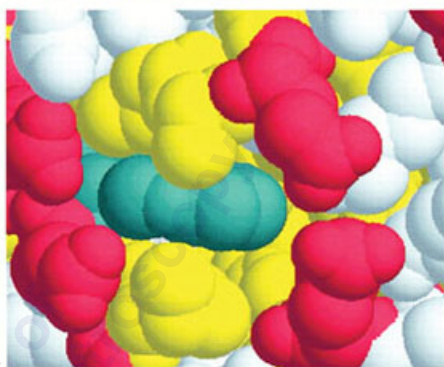
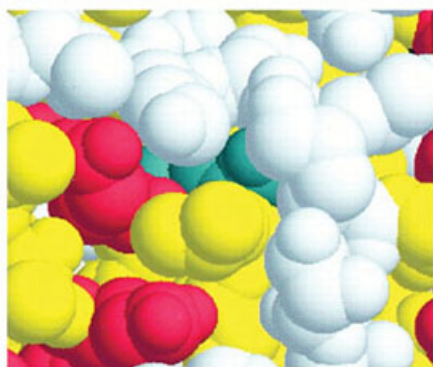
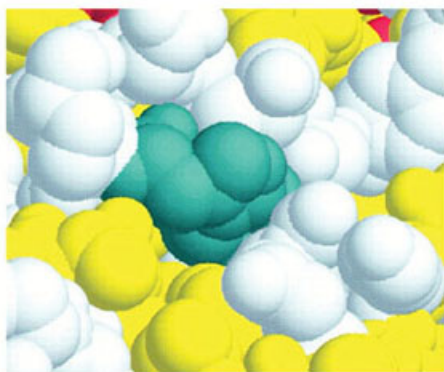
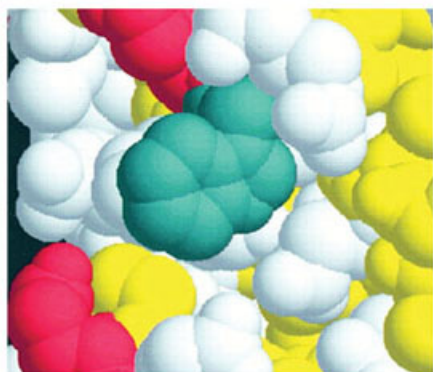
W189





W49

W189

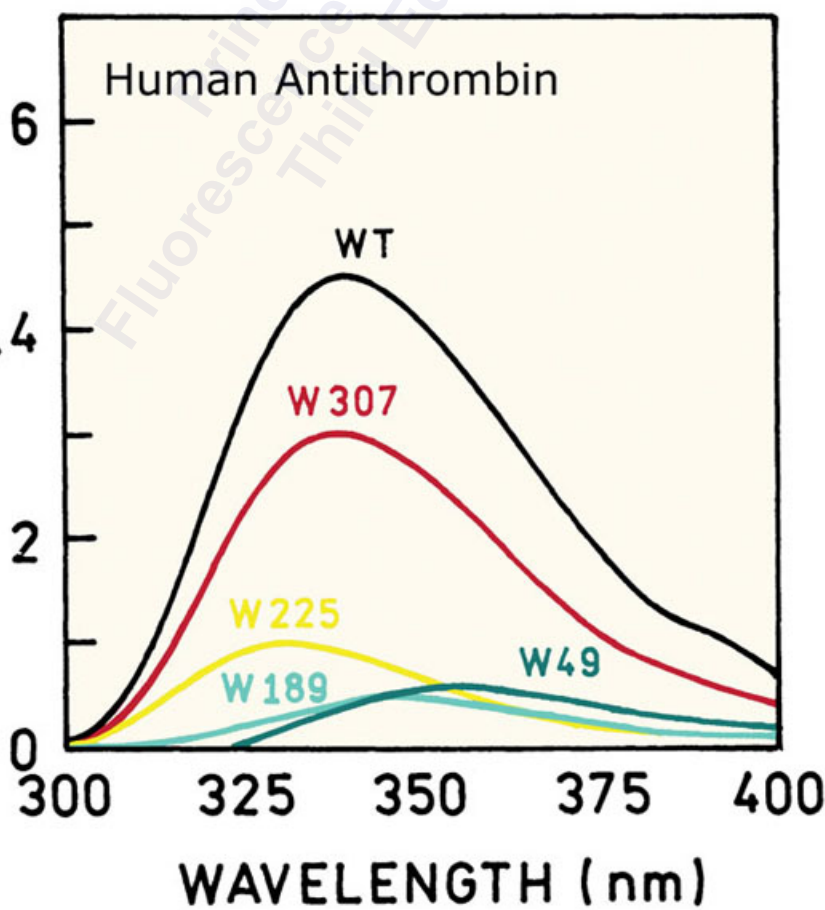


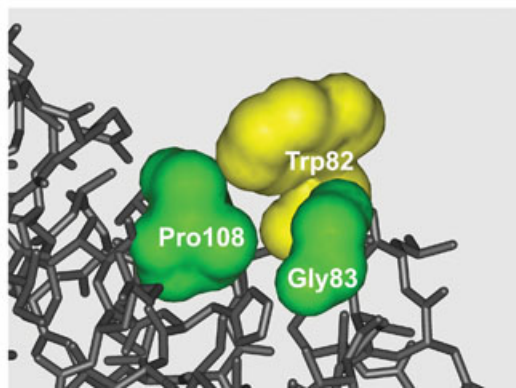
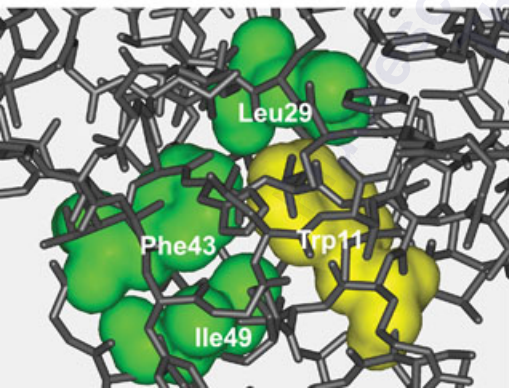
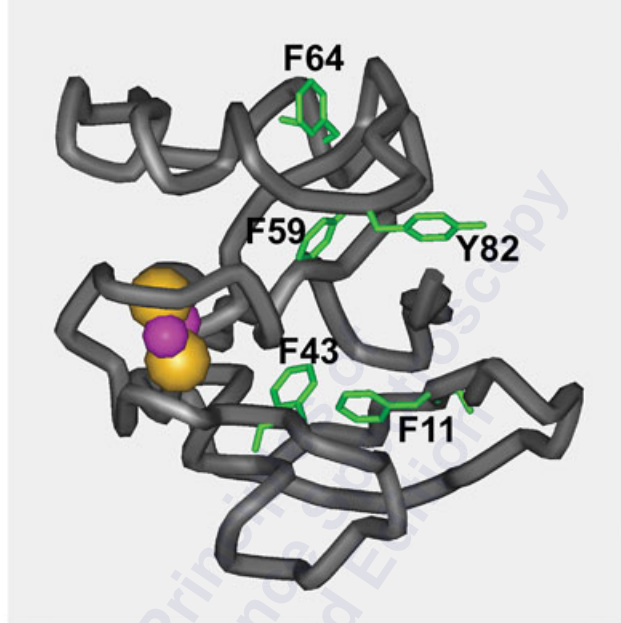
W225

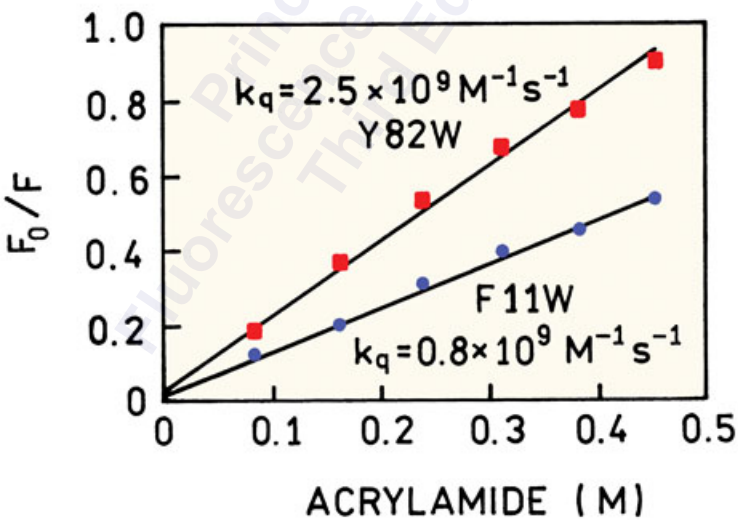
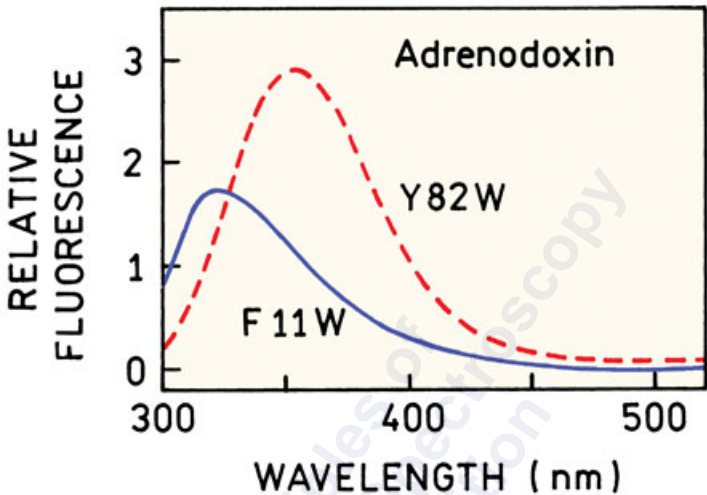
W307

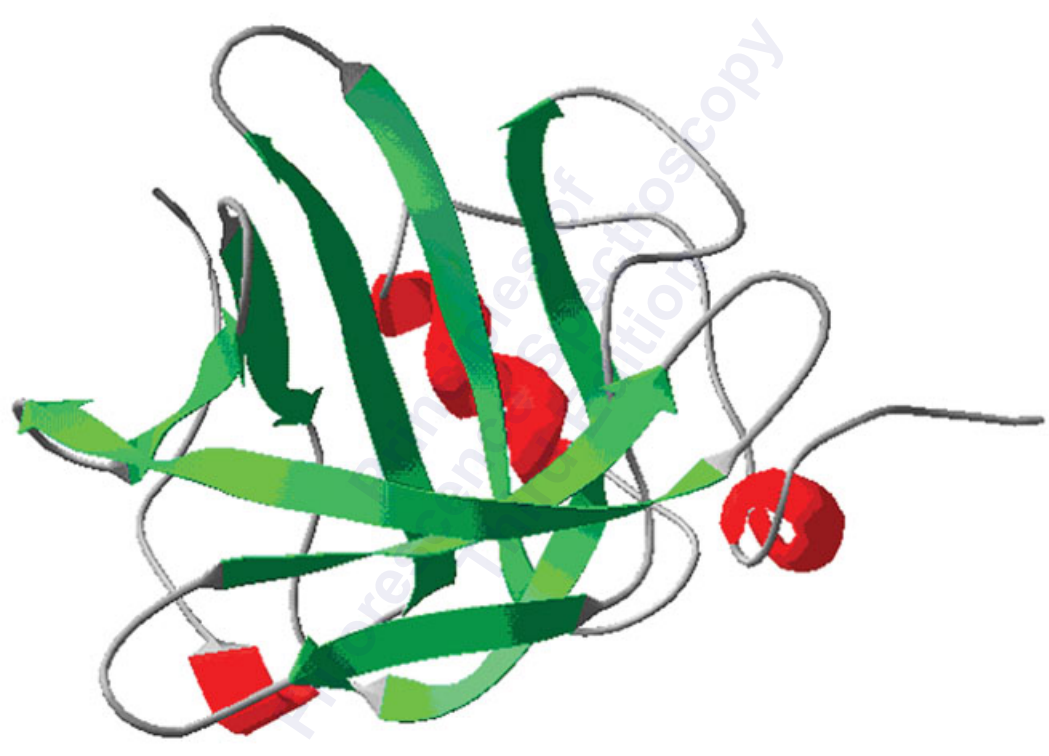
FLUORESCENCE INTENSITY

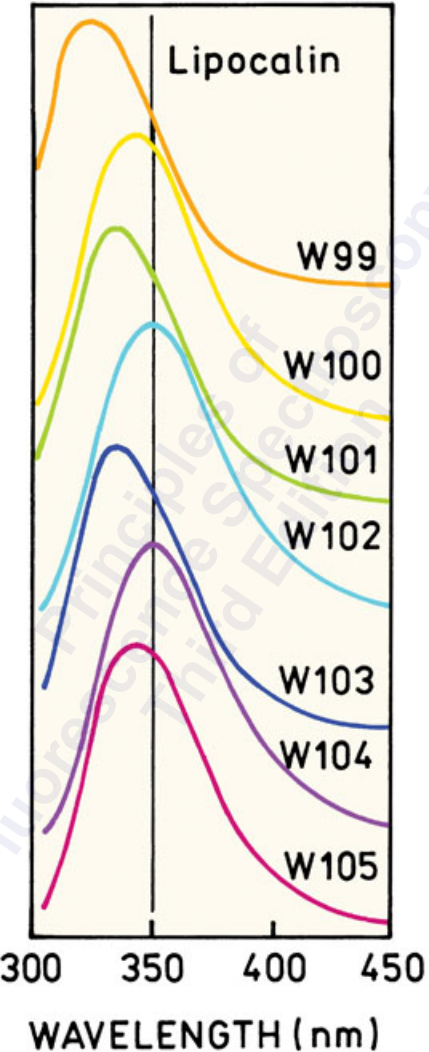
Human Antithrombin

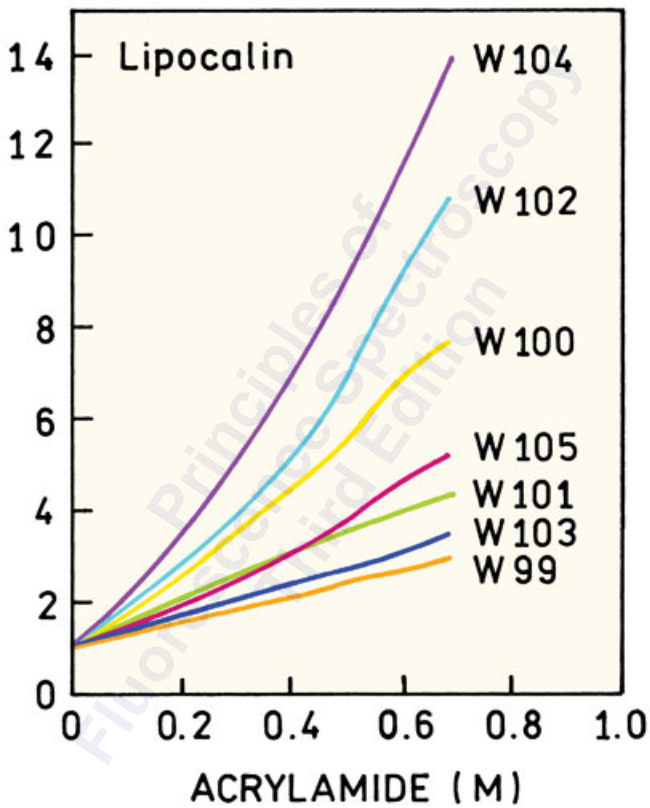






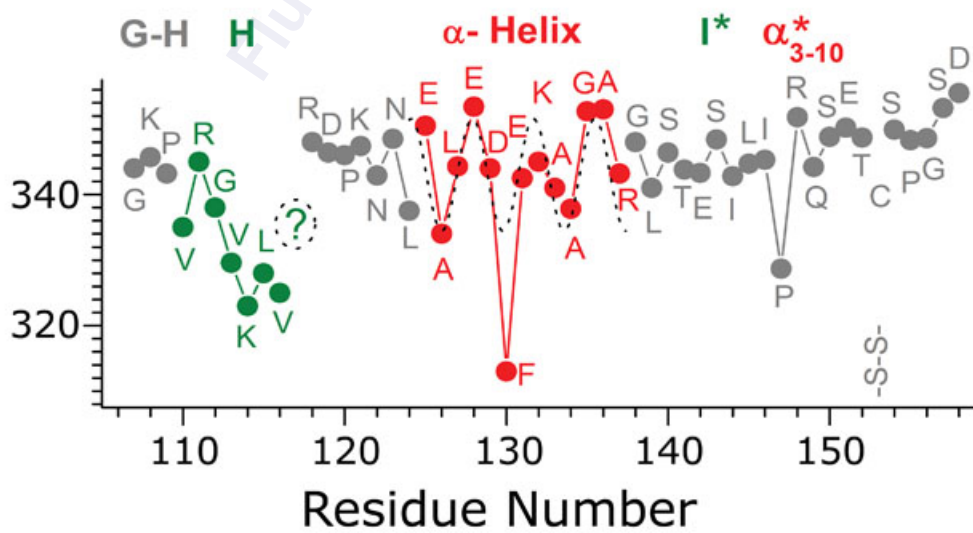
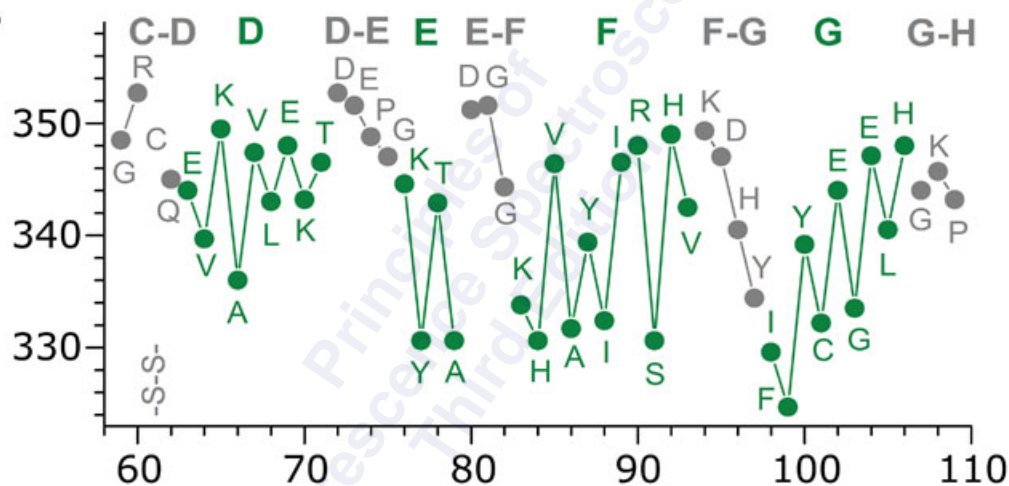
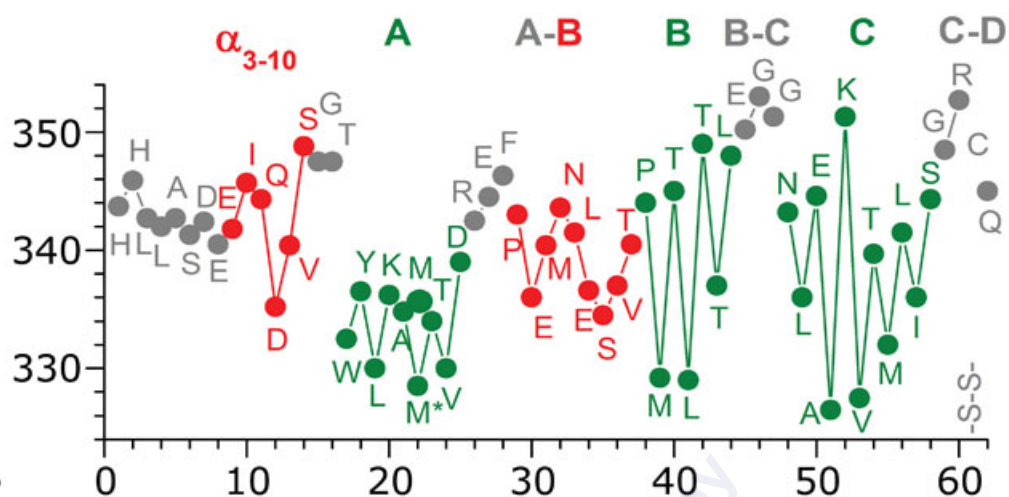


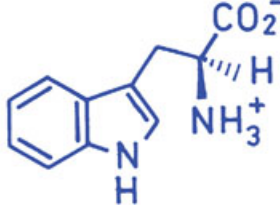




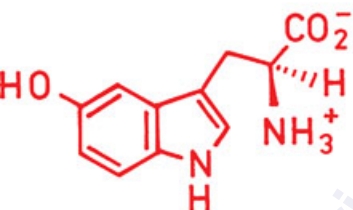


Emission Maximum (nm)

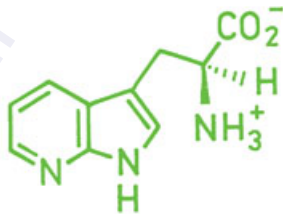




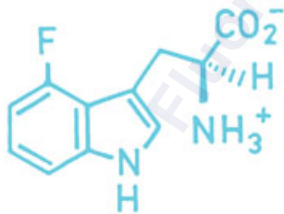
W (Trp)  $Q=0.13$



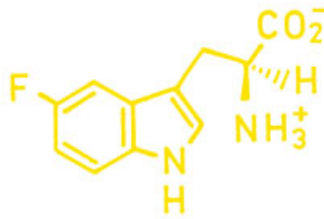
5HW  $Q=0.26$



7AW  $Q=0.02$



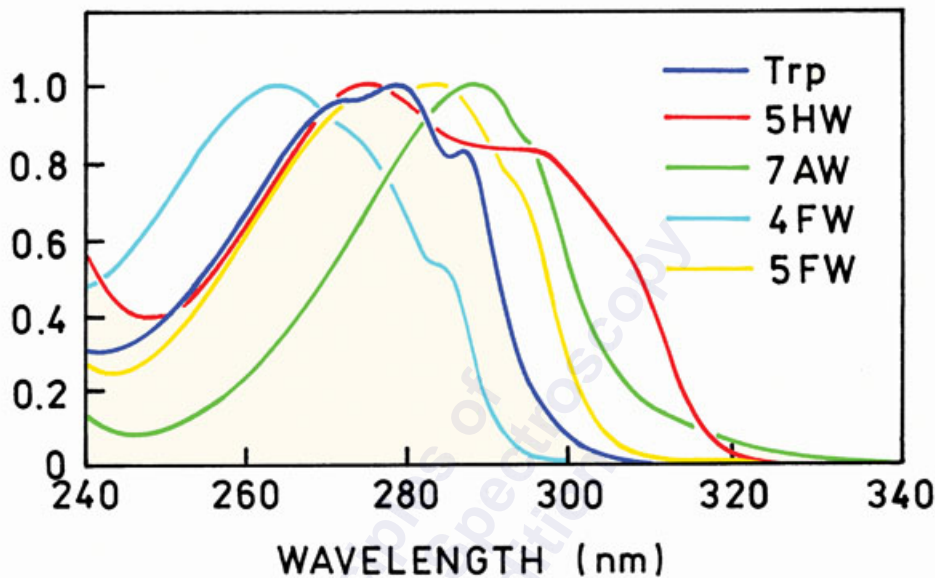
4FW  $Q=0.001$



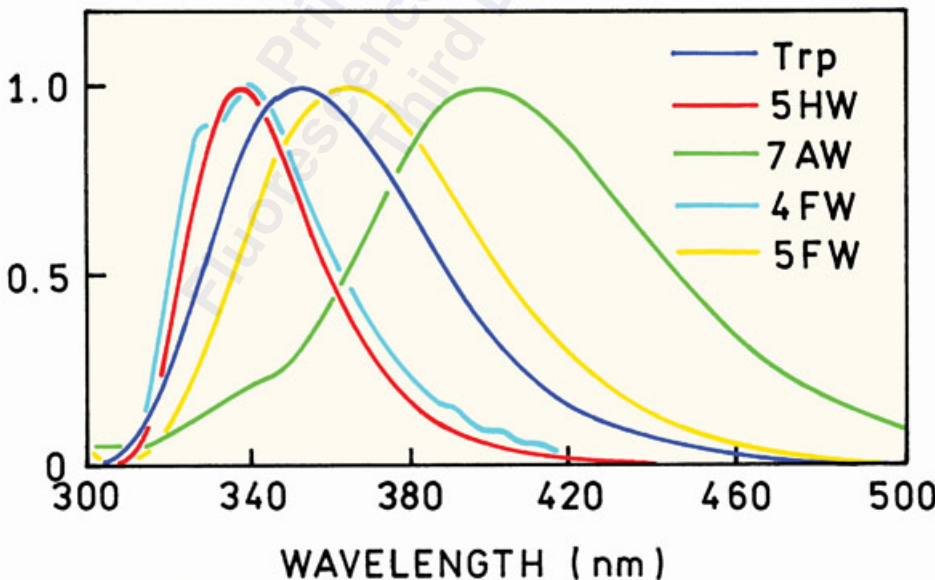
5FW  $Q=0.14$



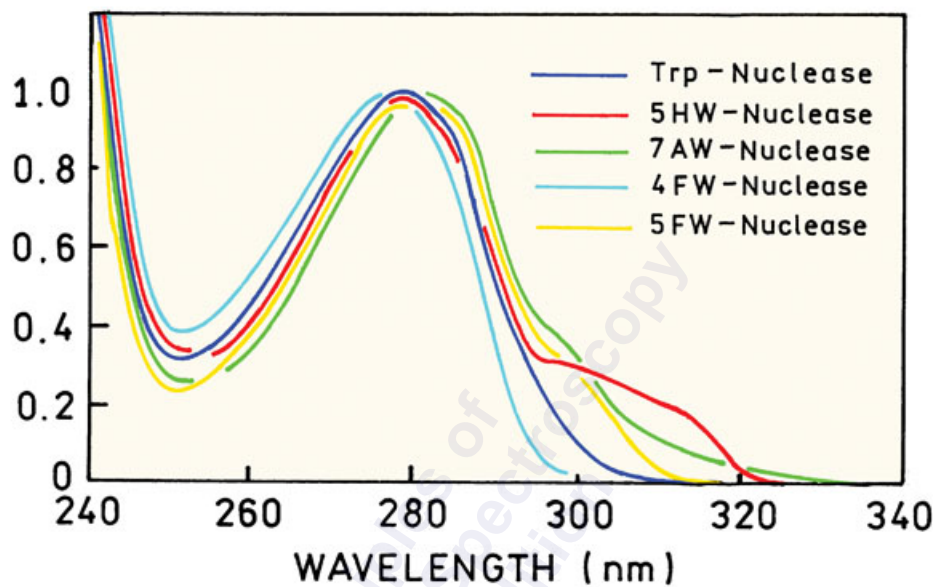
ABSORBANCE



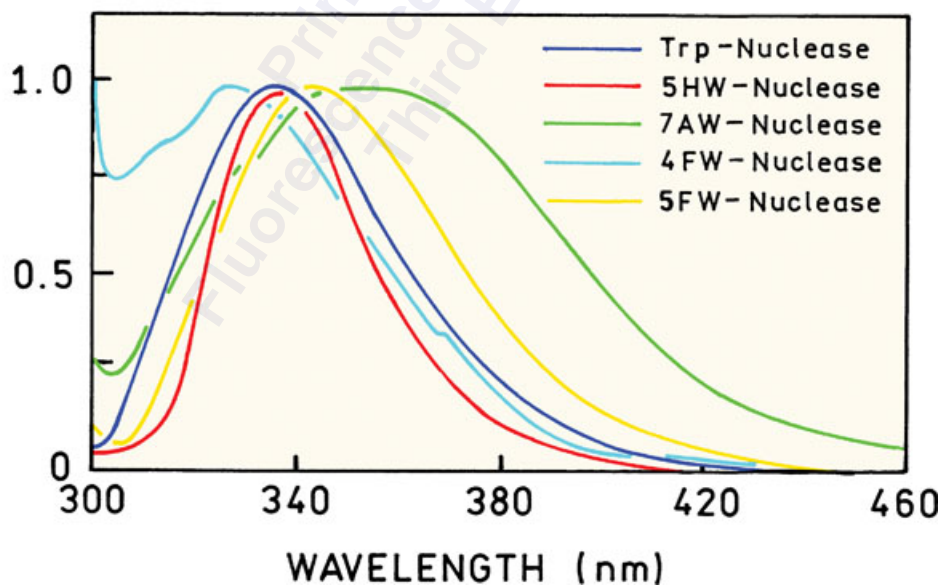
FLUORESCENCE

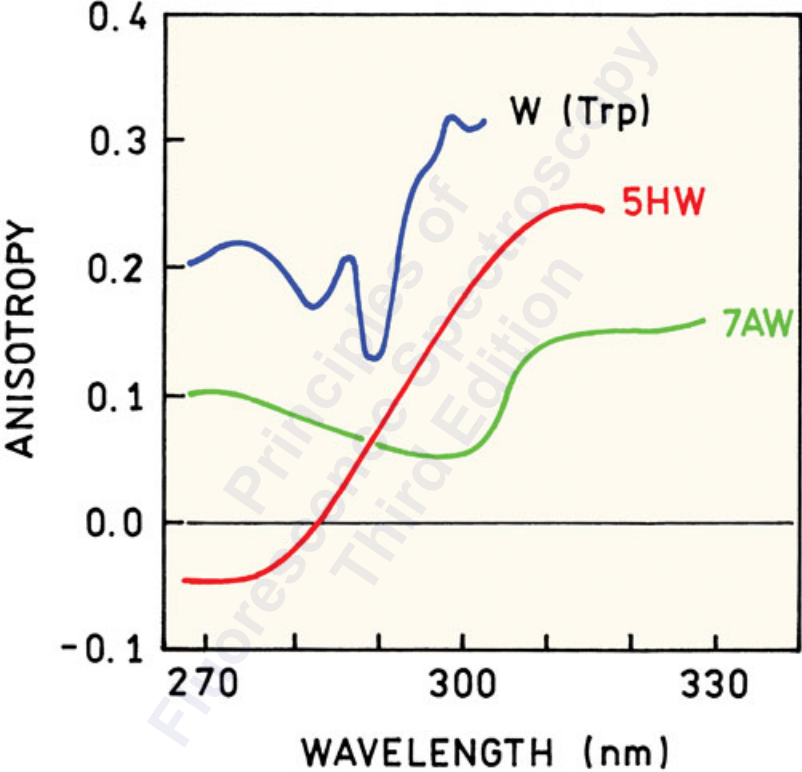


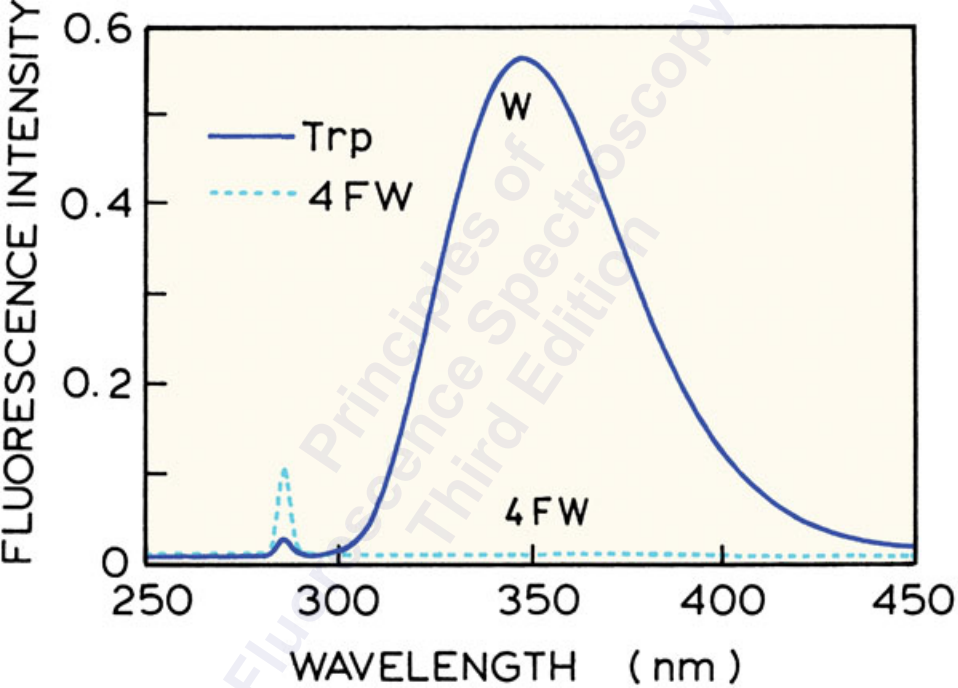
ABSORBANCE

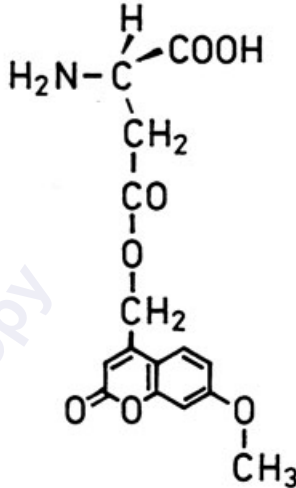
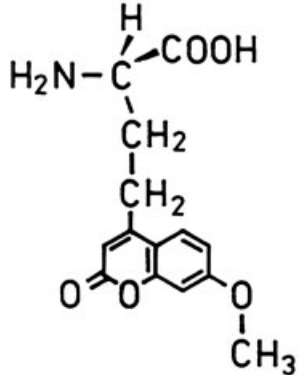
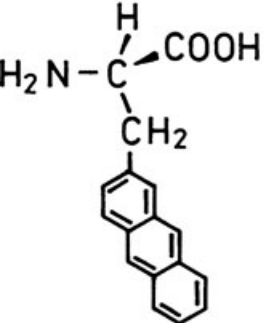


FLUORESCENCE

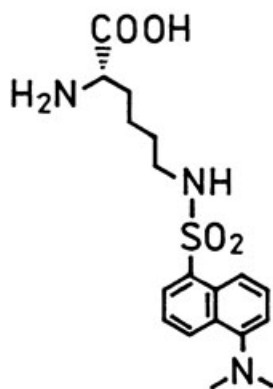
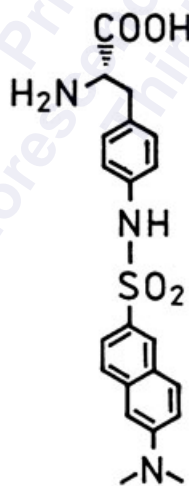
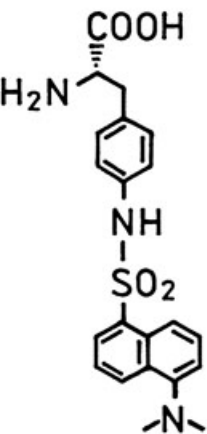




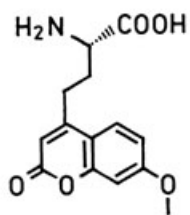




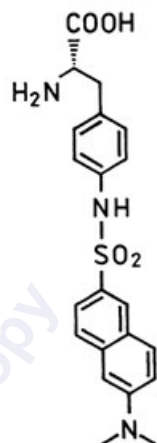
mchAla



2,6-dnsAF



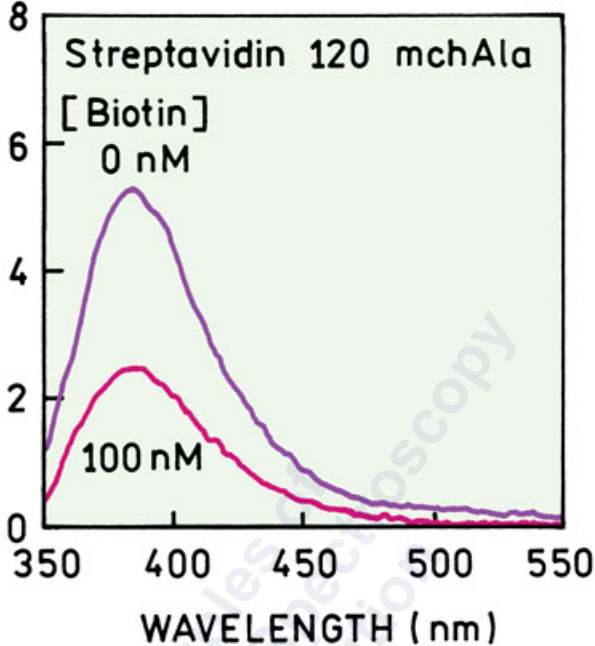
**mchAla**



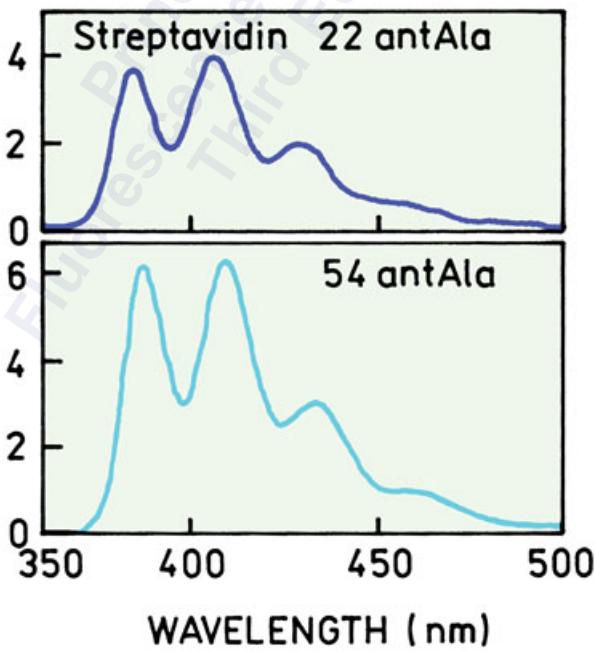
**2,6-dnsAF**



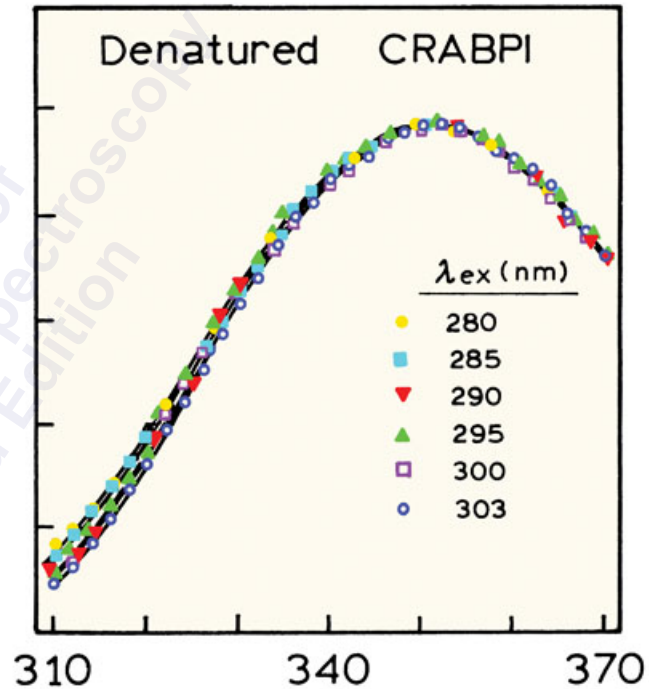
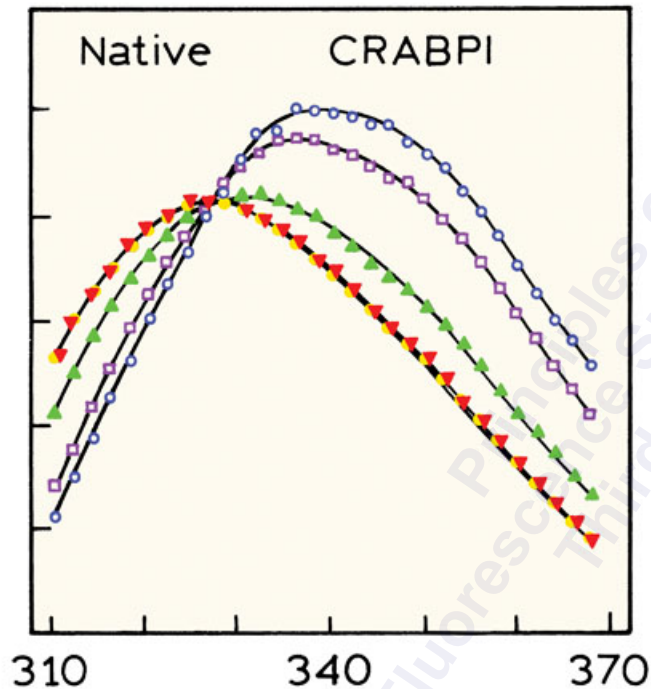
FLUORESCENCE INTENSITY



FLUORESCENCE INTENSITY

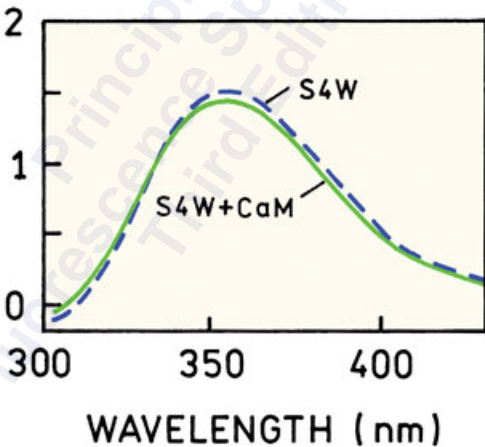
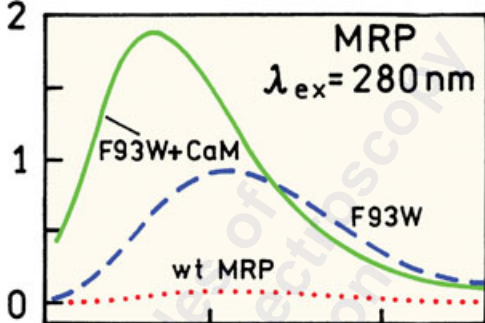


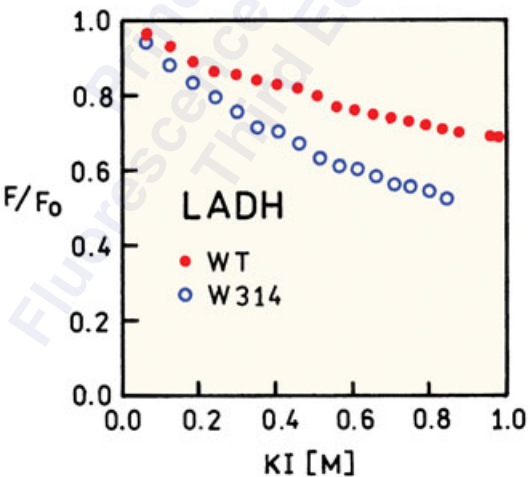
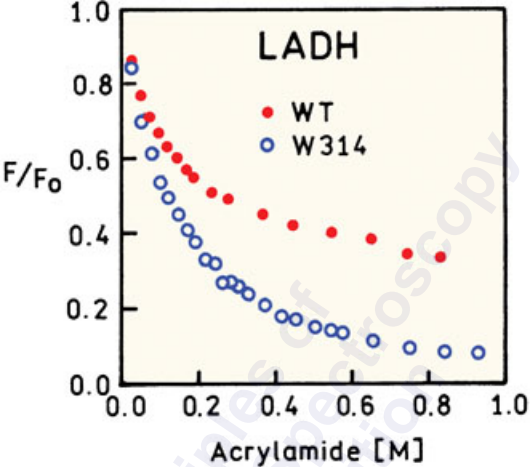
FLUORESCENCE INTENSITY

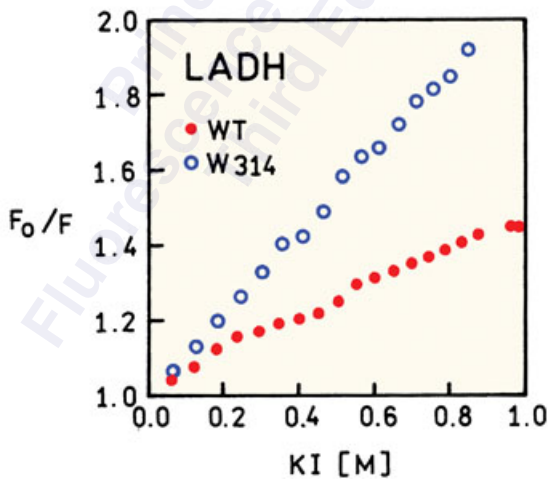
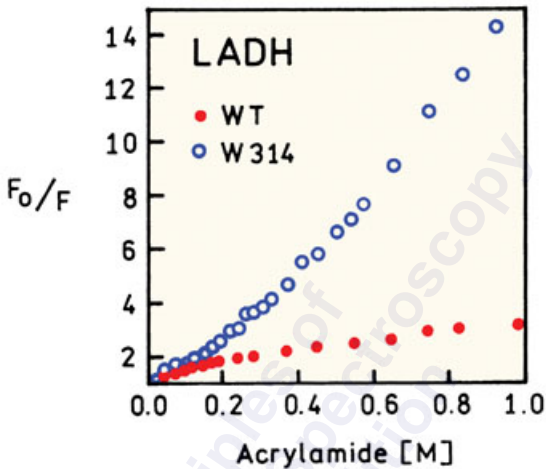


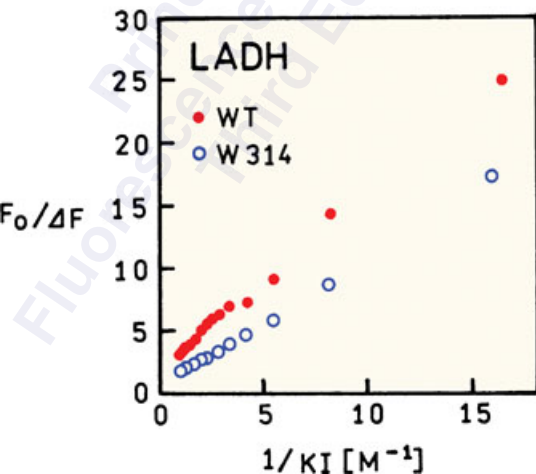
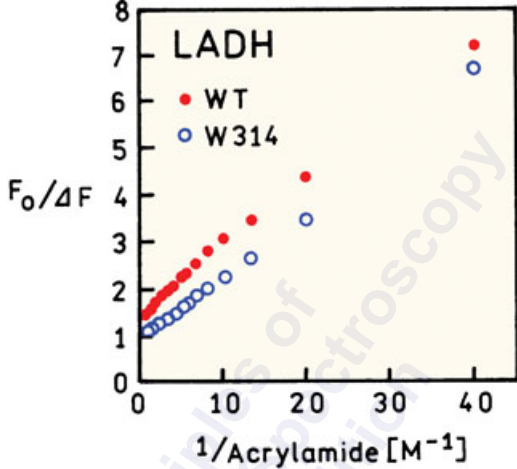


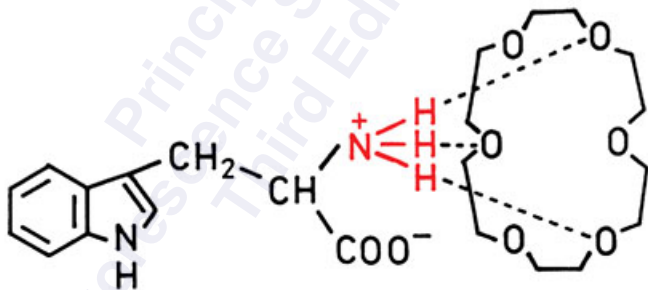
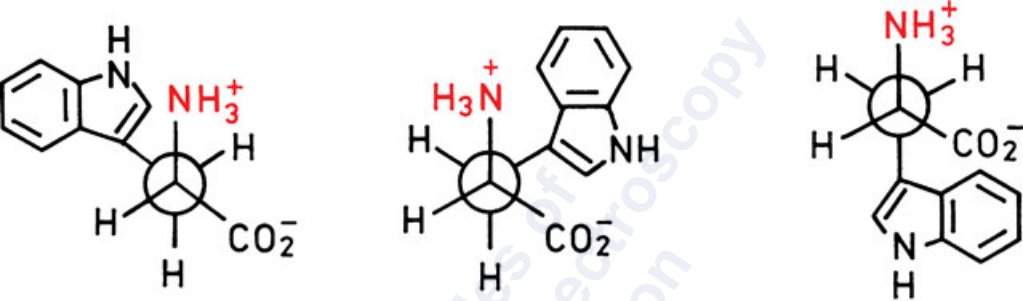
RELATIVE FLUORESCENCE



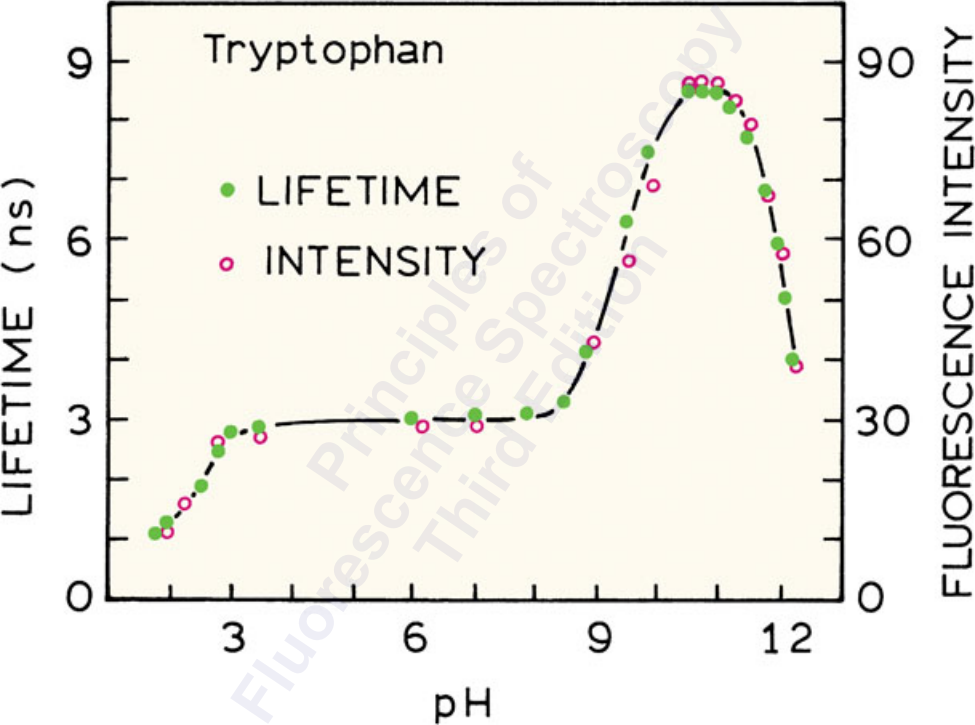


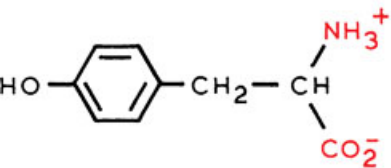




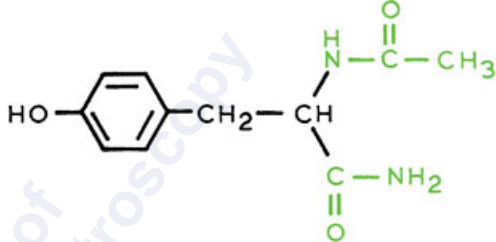


Trp - 18 - Crown - 6

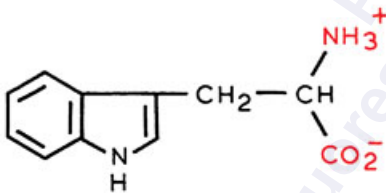




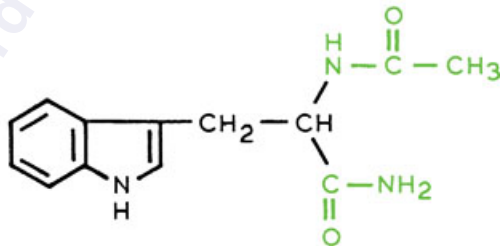
Tyrosine (Tyr, Y)



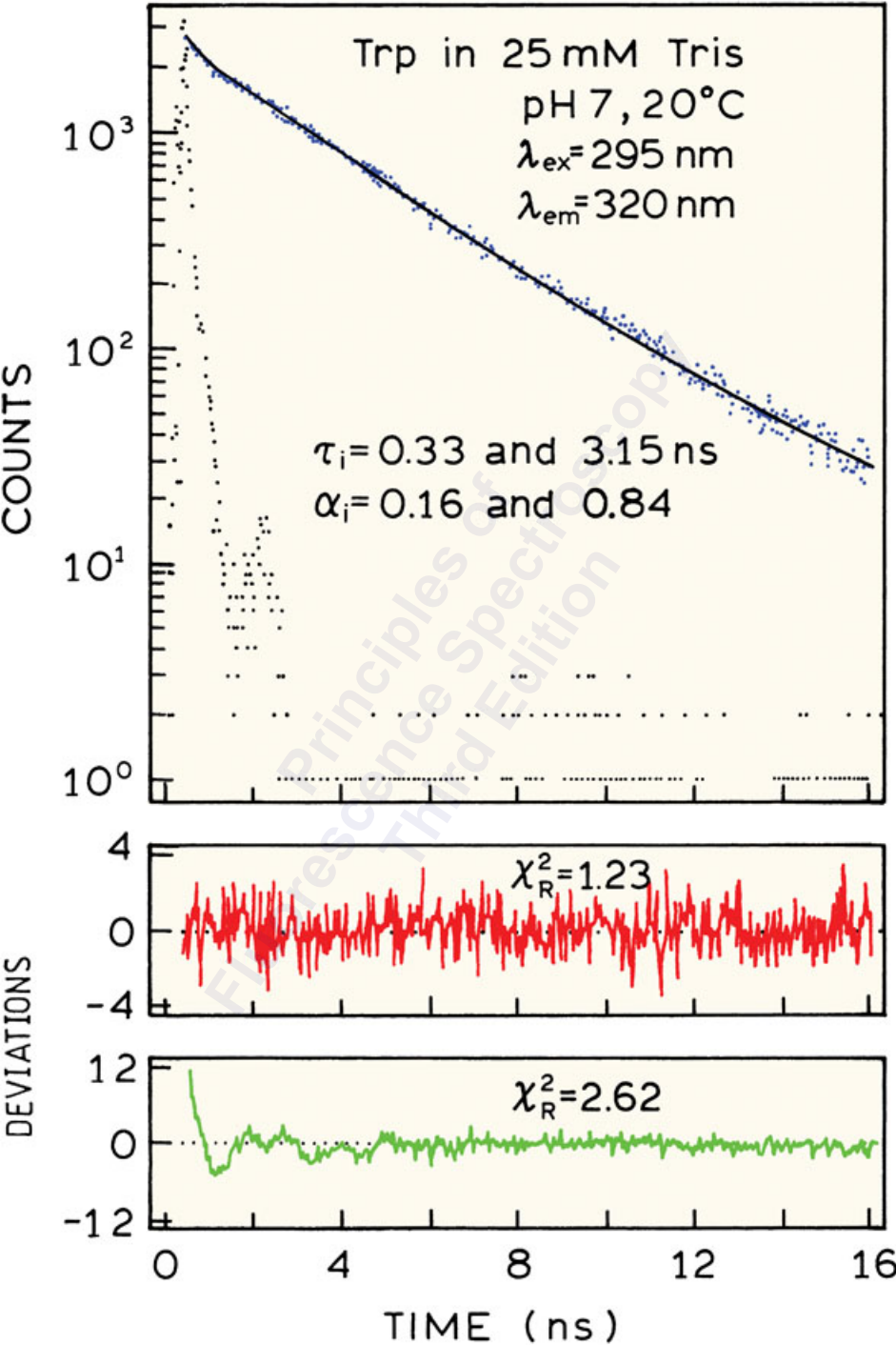
NATyrA



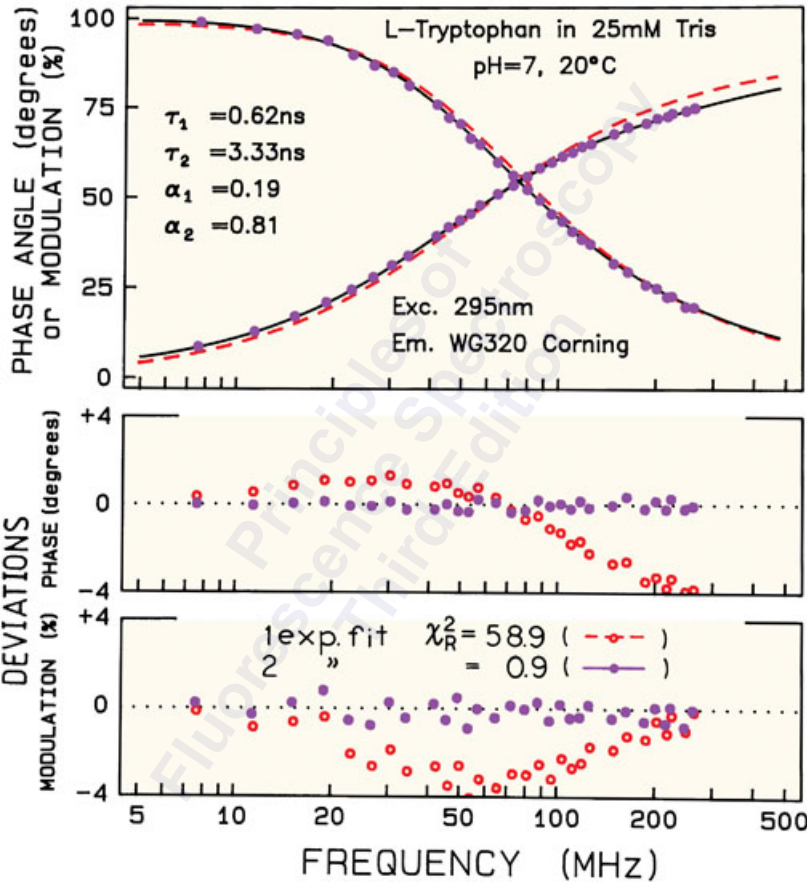
Tryptophan (Trp, W)

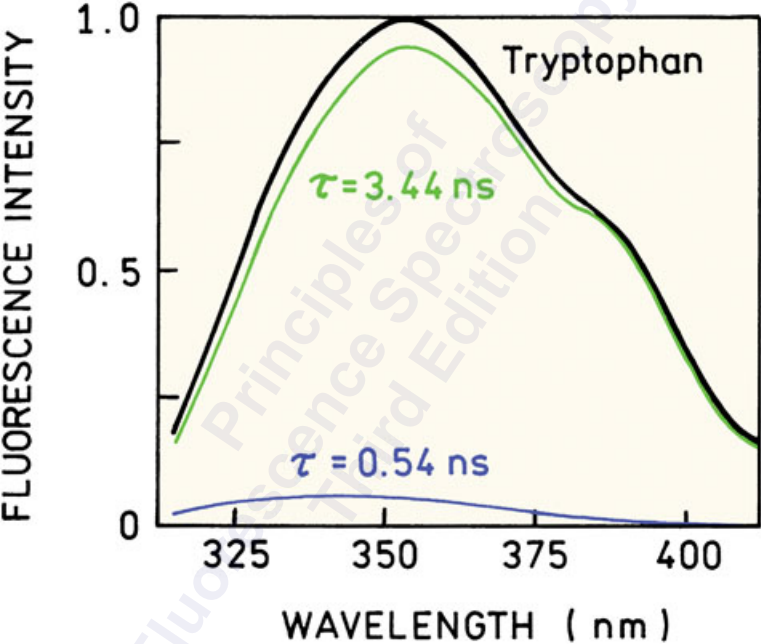


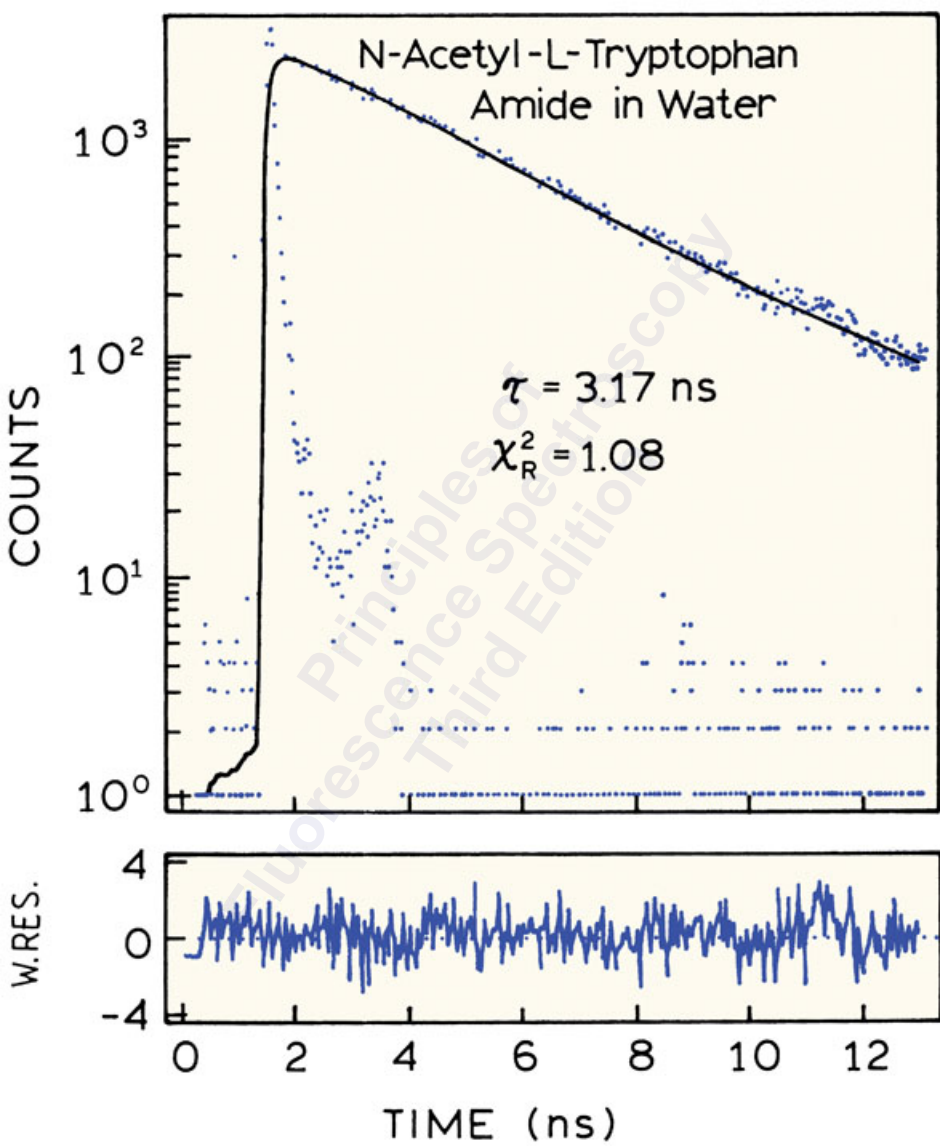
NATA

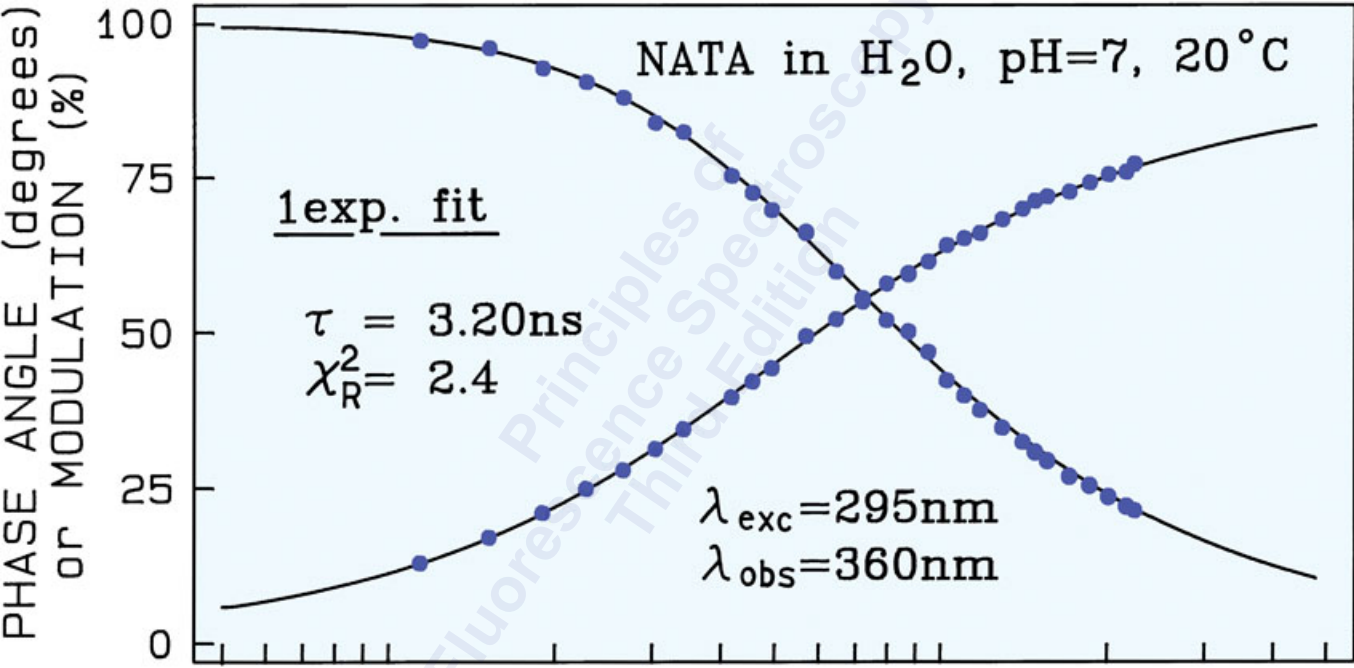


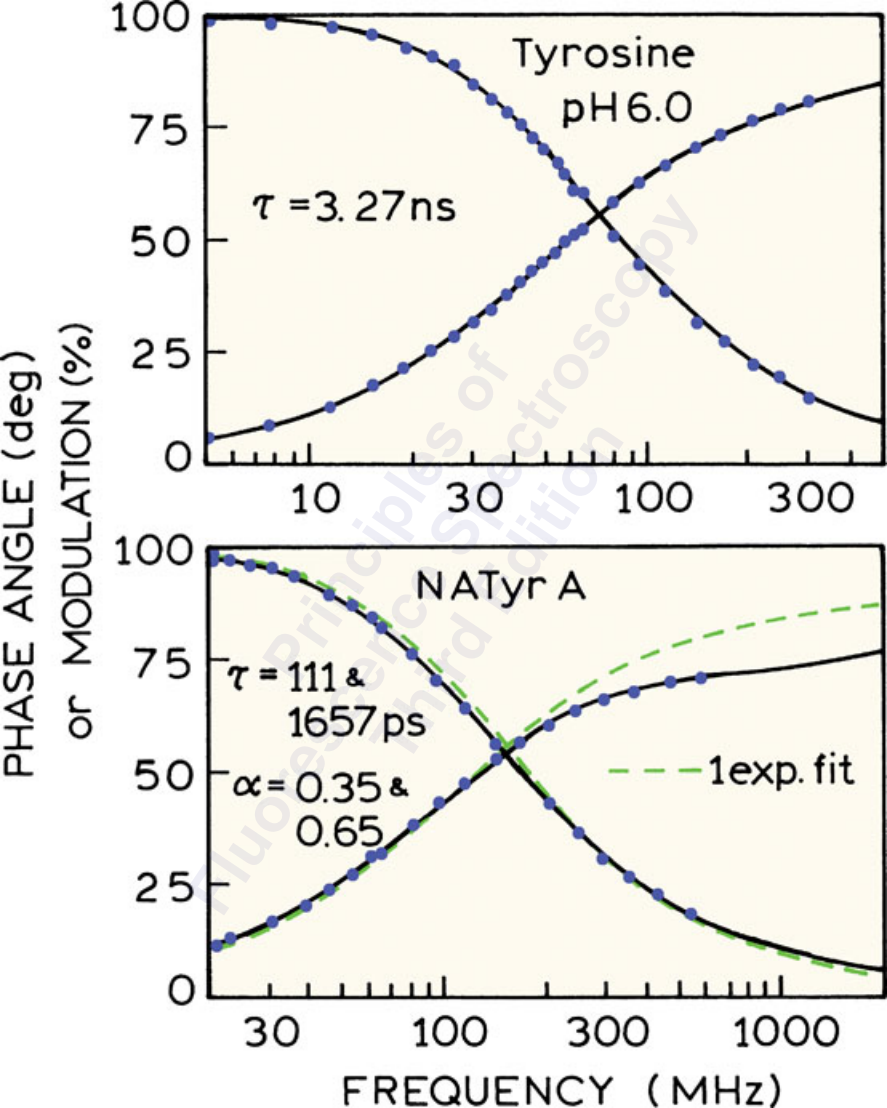


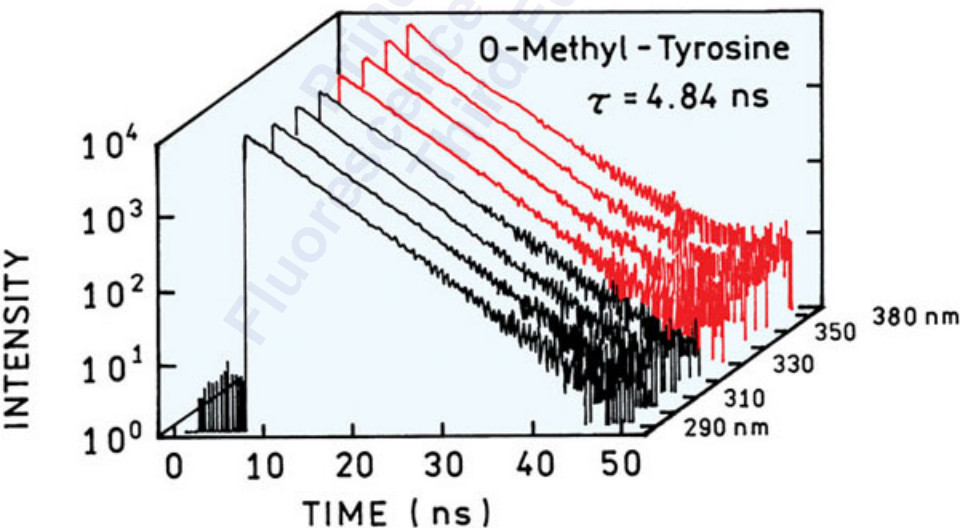
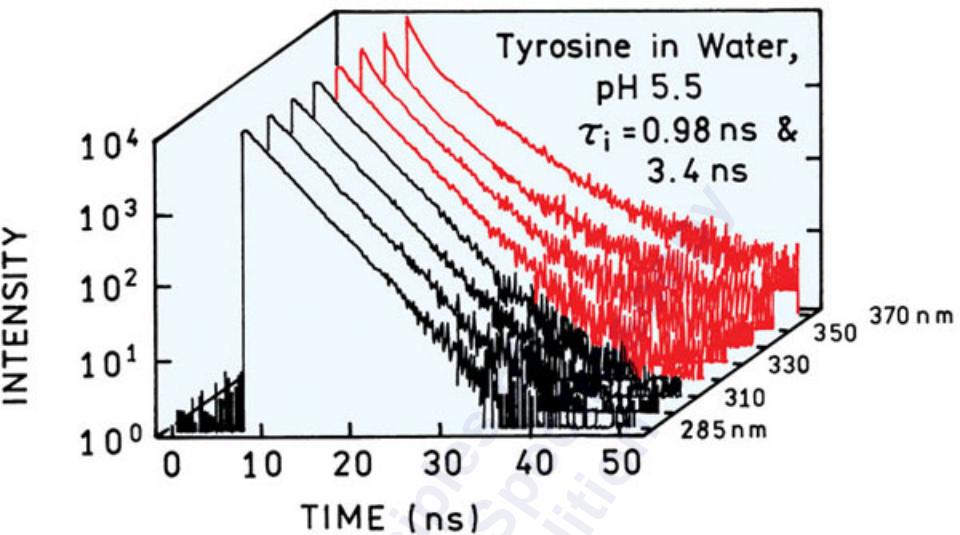


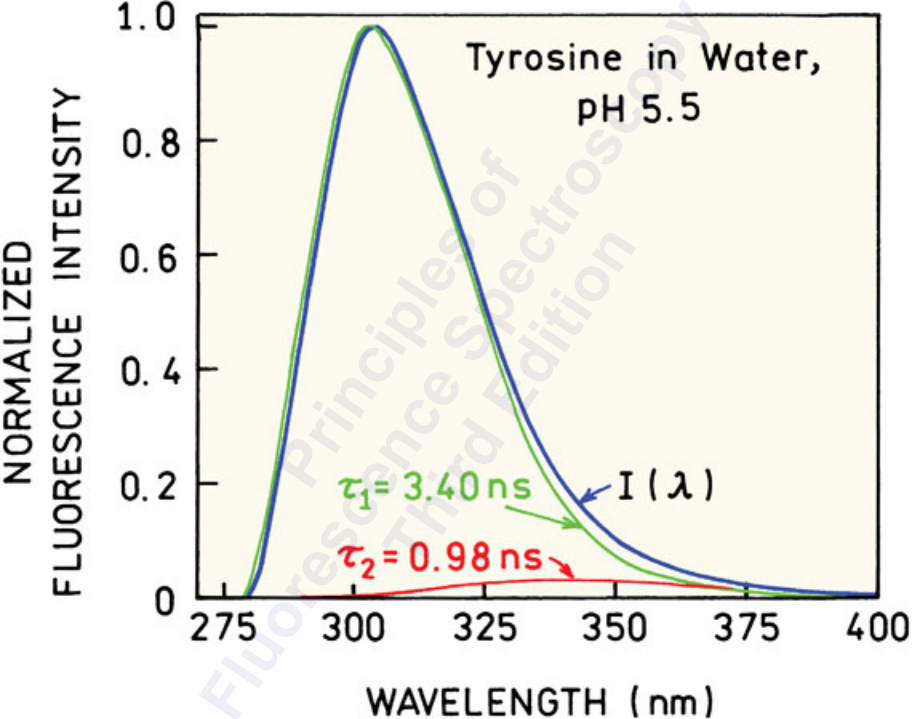


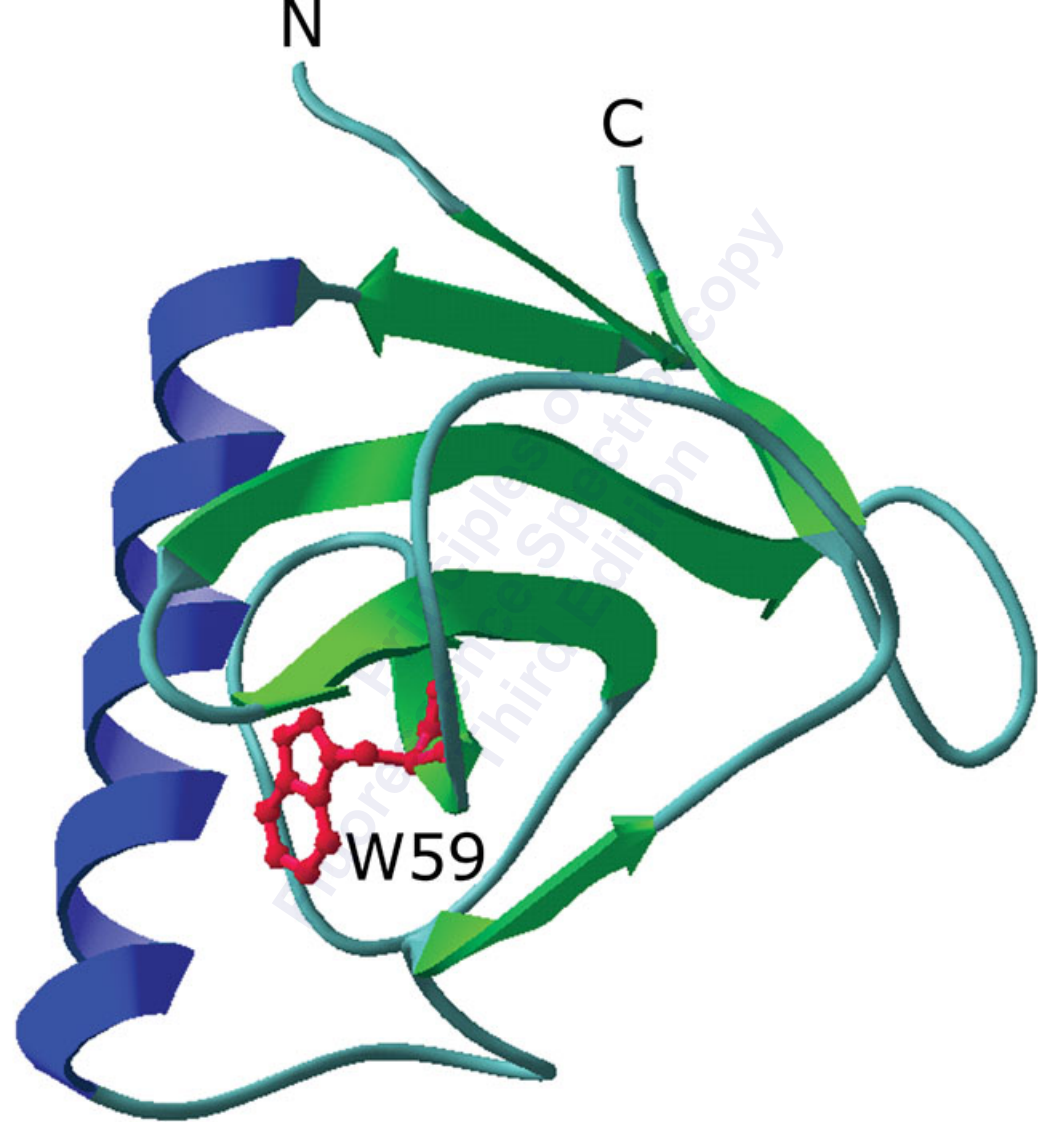






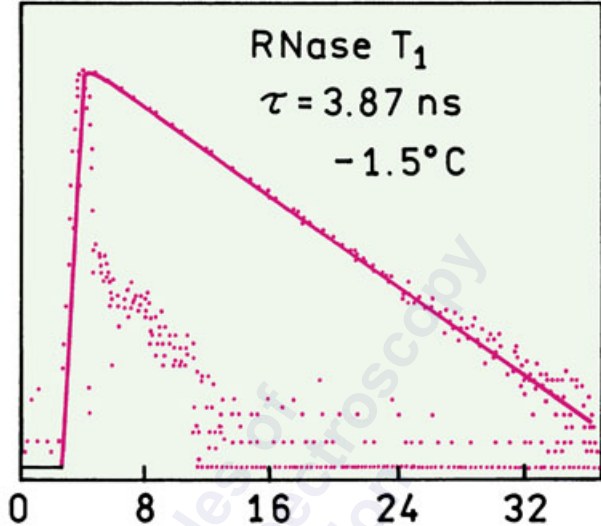




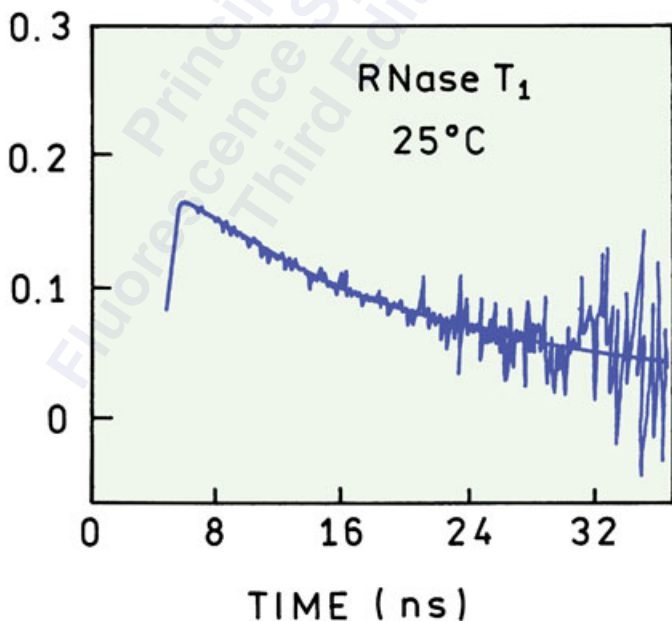




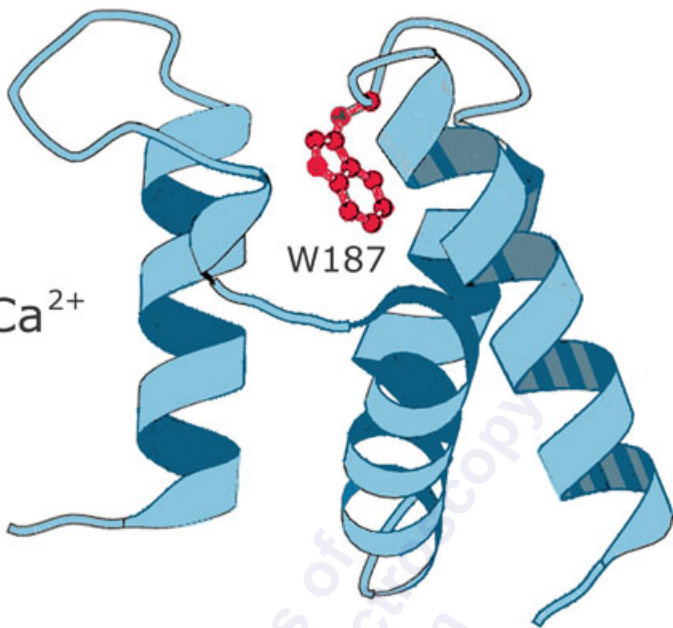
log INTENSITY



ANISOTROPY

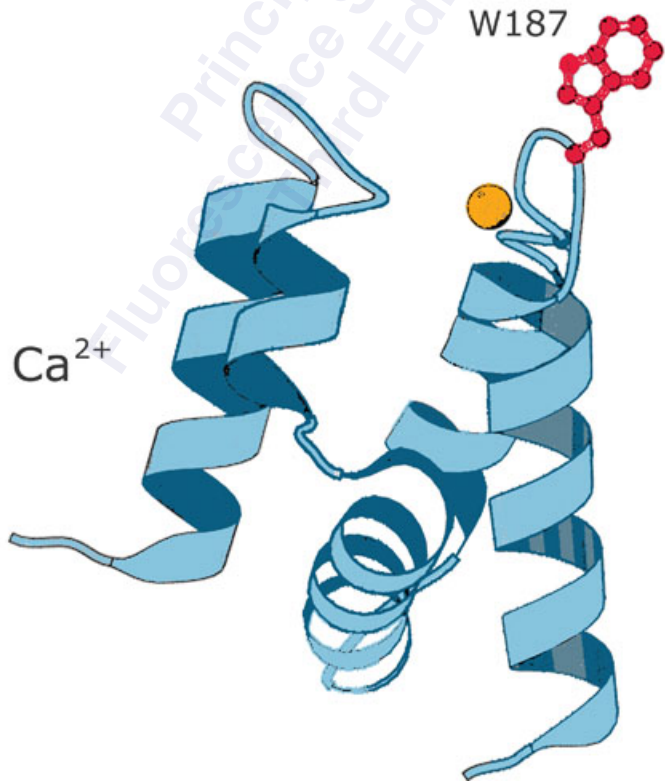


No  $\text{Ca}^{2+}$

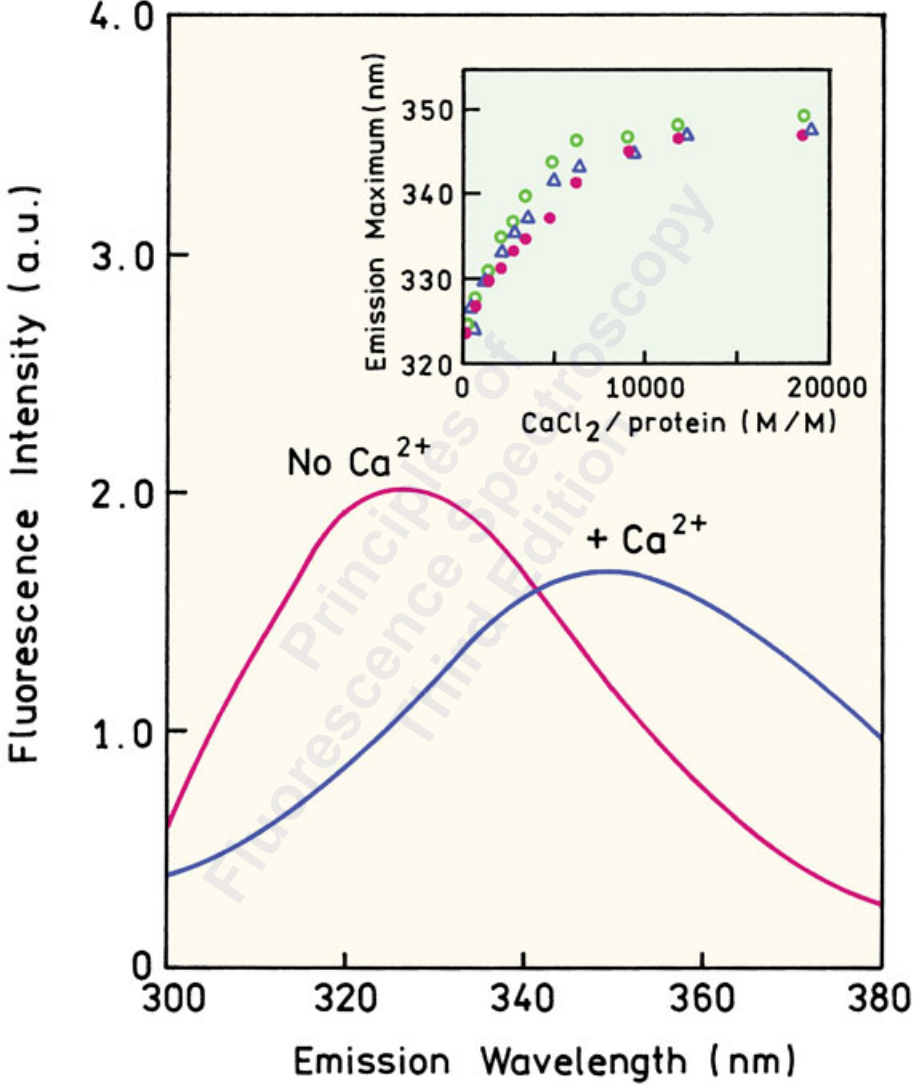


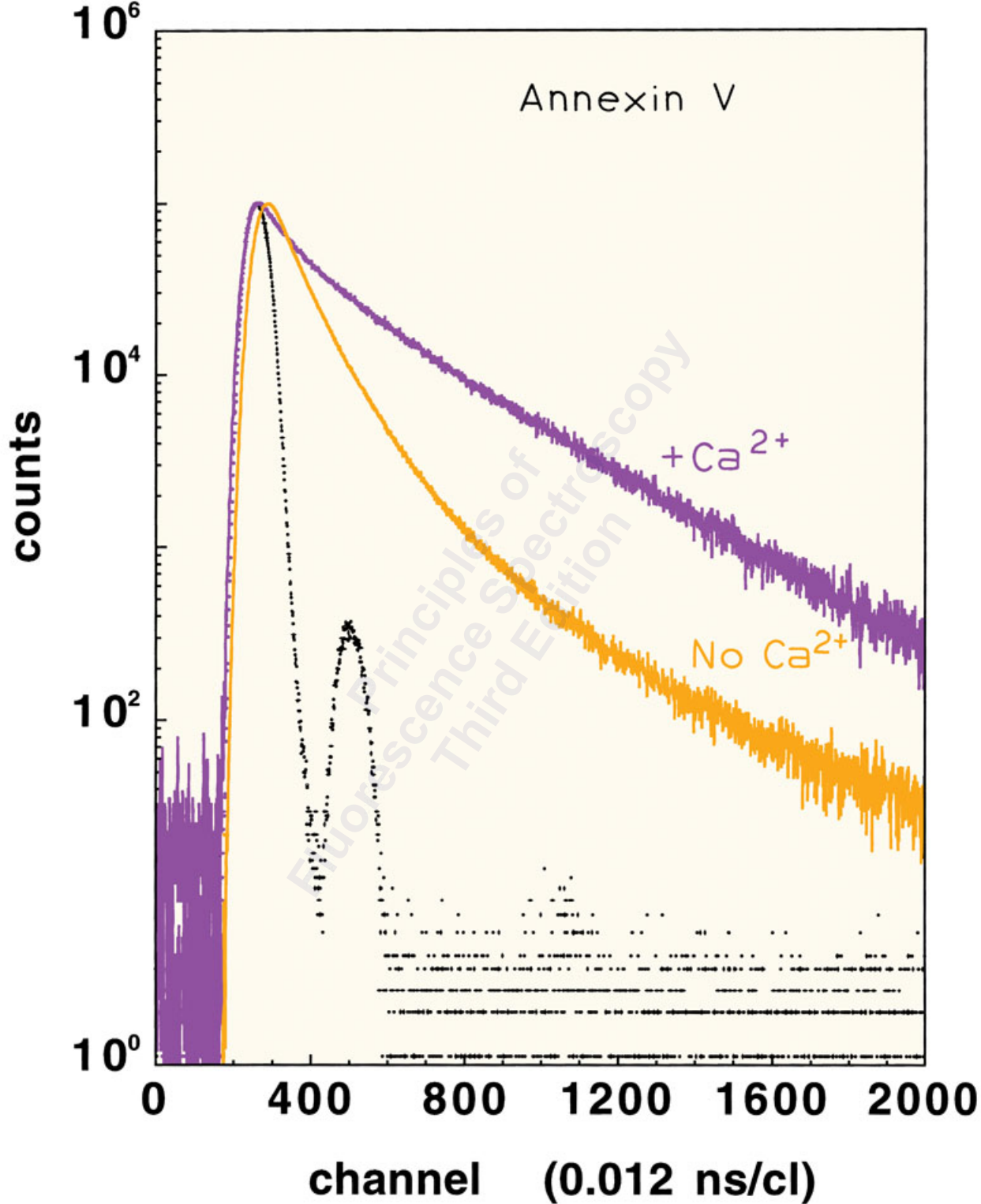
W187

+  $\text{Ca}^{2+}$

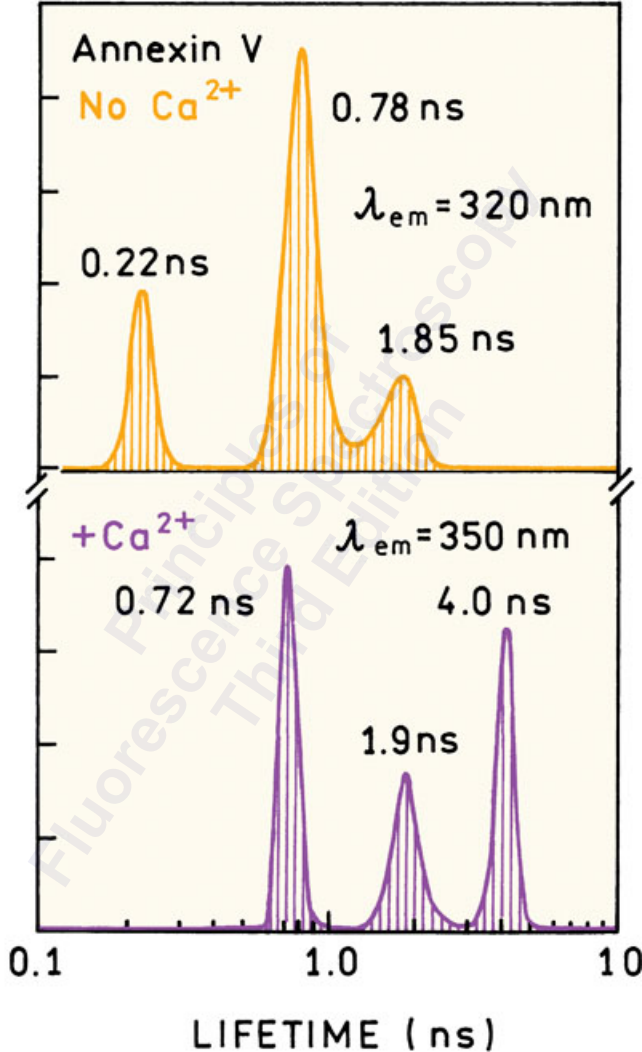


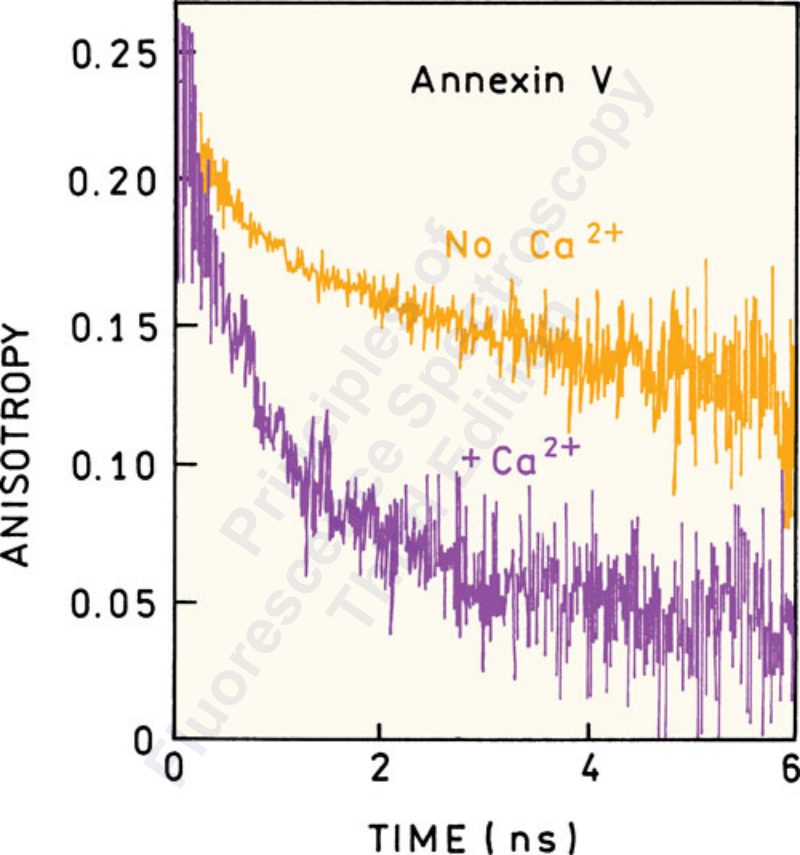
W187



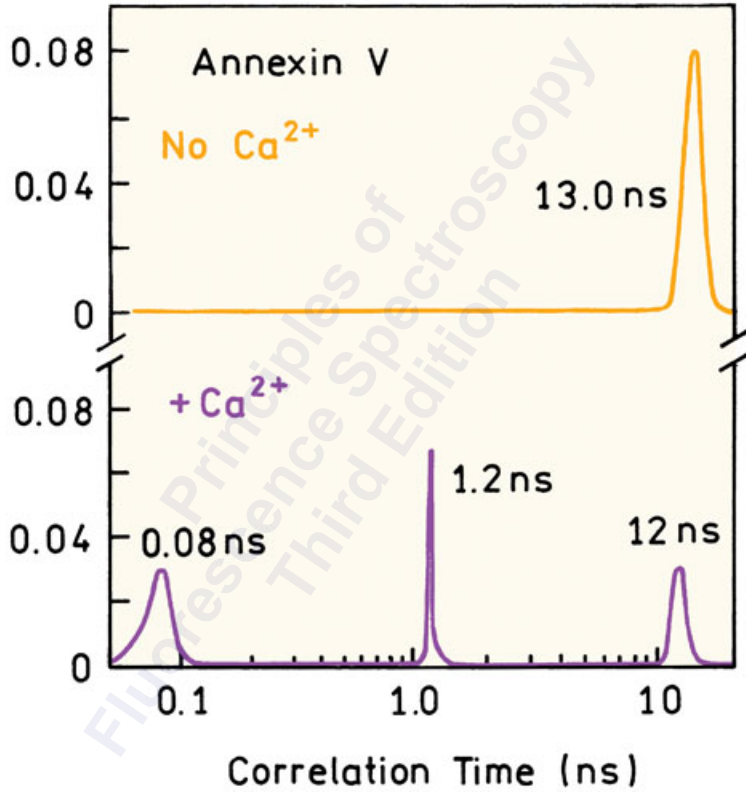


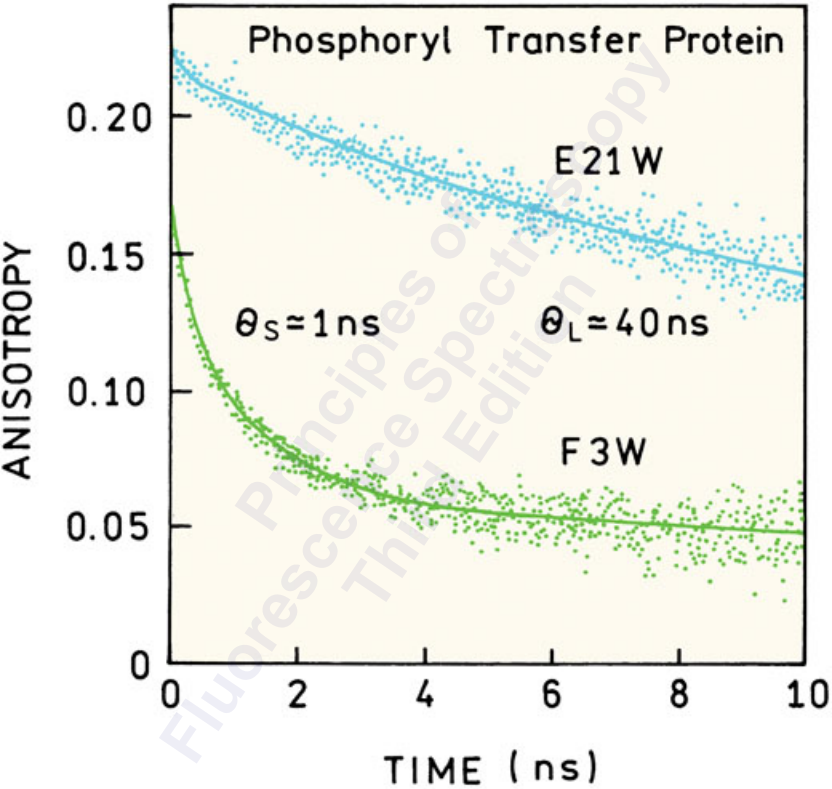
$\propto I(t)$





Anisotropy







FLUORESCENCE INTENSITY

RNase T<sub>1</sub>, pH=5.5

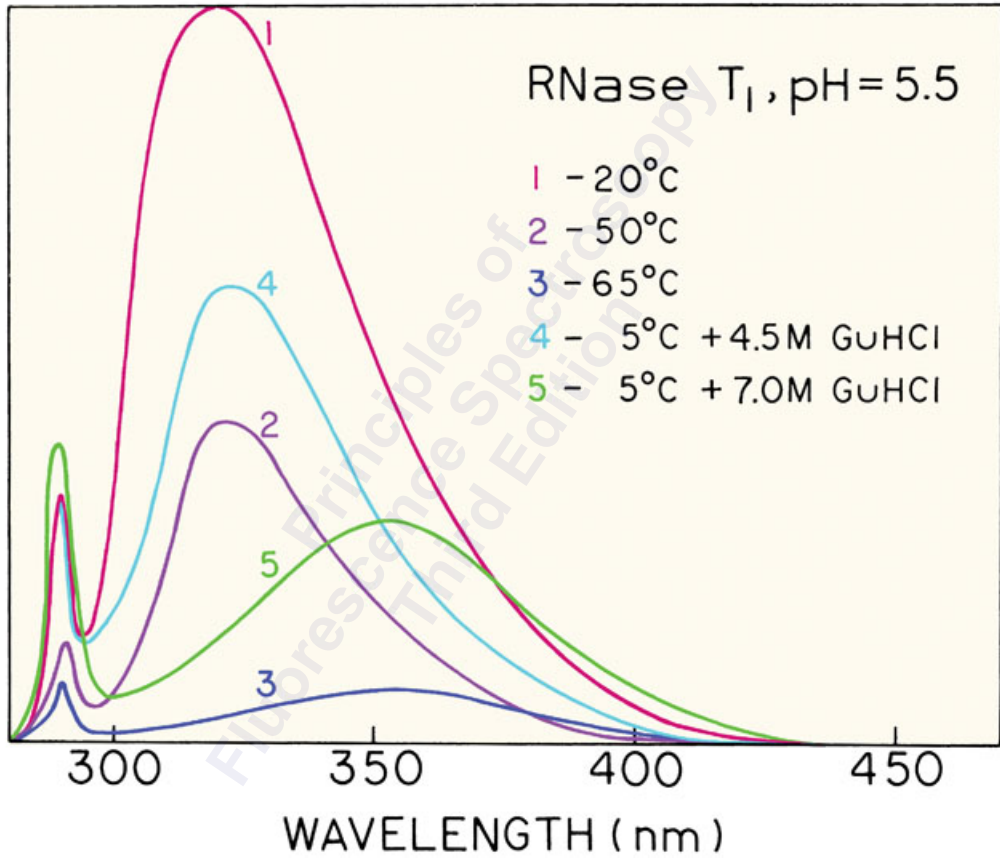
1 - 20°C

2 - 50°C

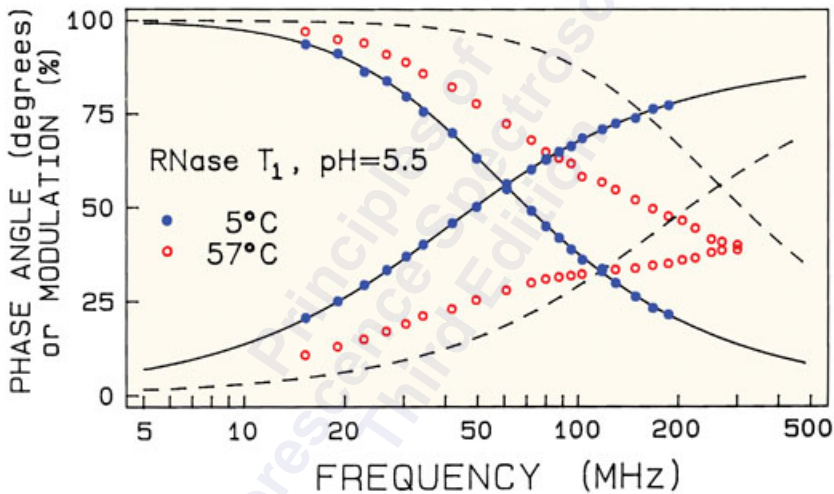
3 - 65°C

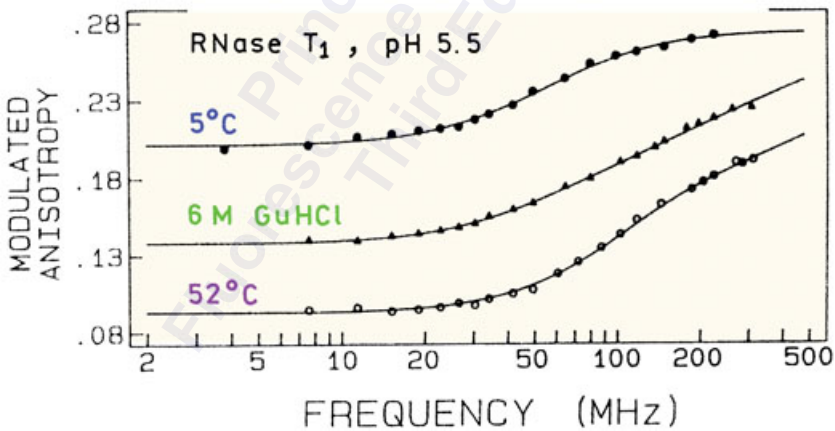
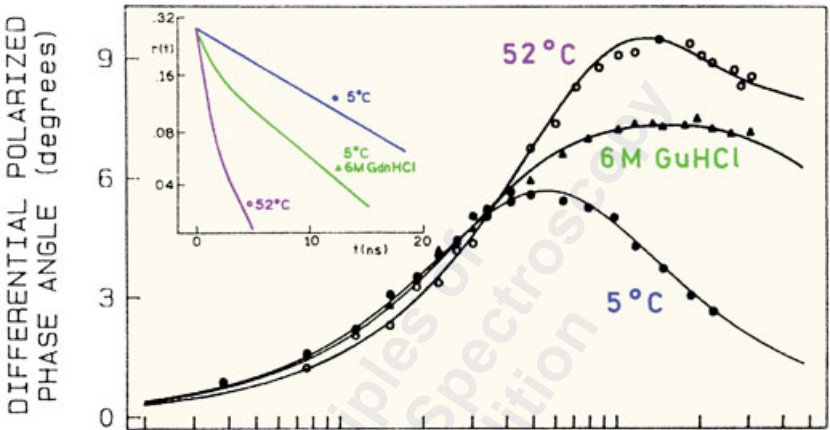
4 - 5°C + 4.5M GuHCl

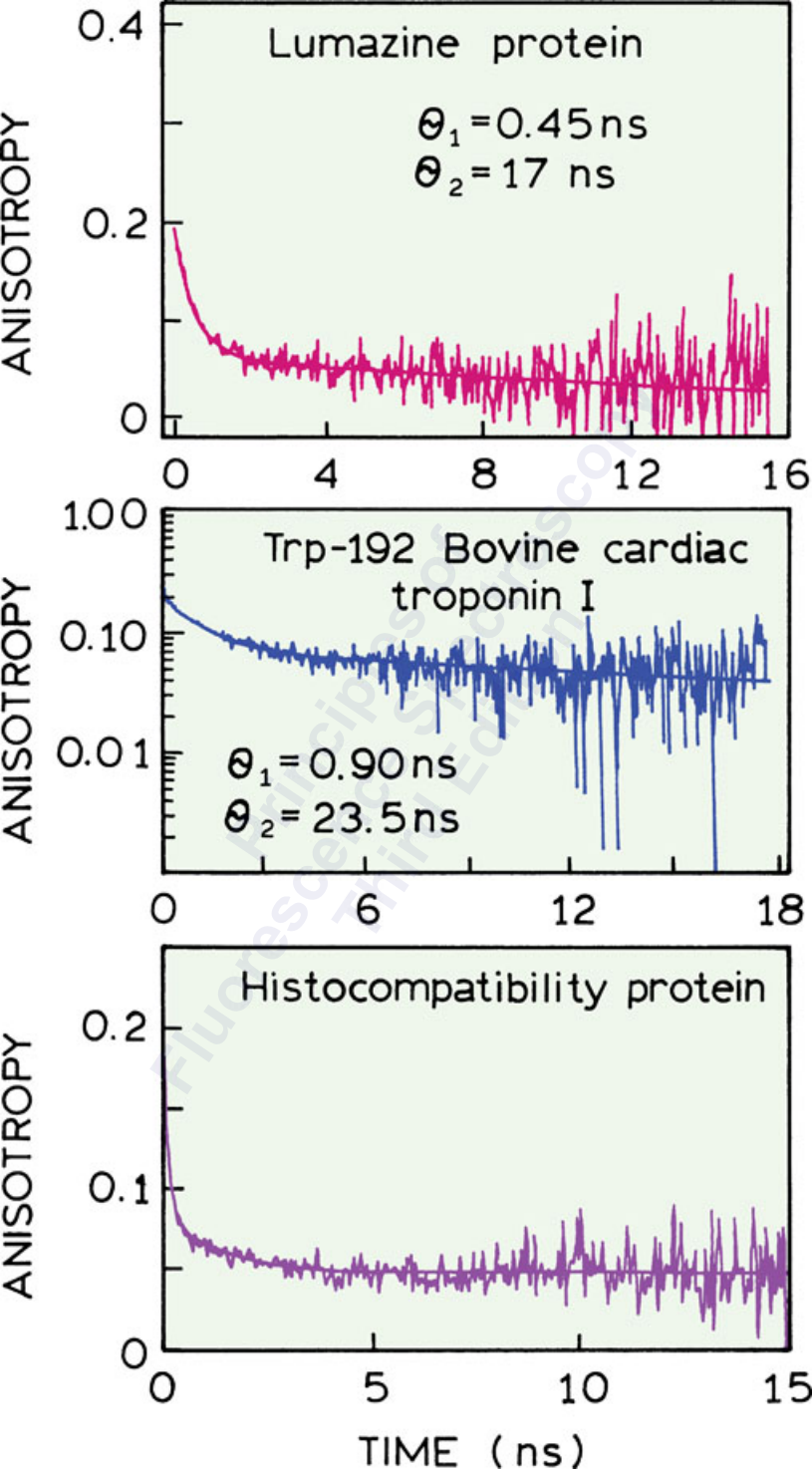
5 - 5°C + 7.0M GuHCl

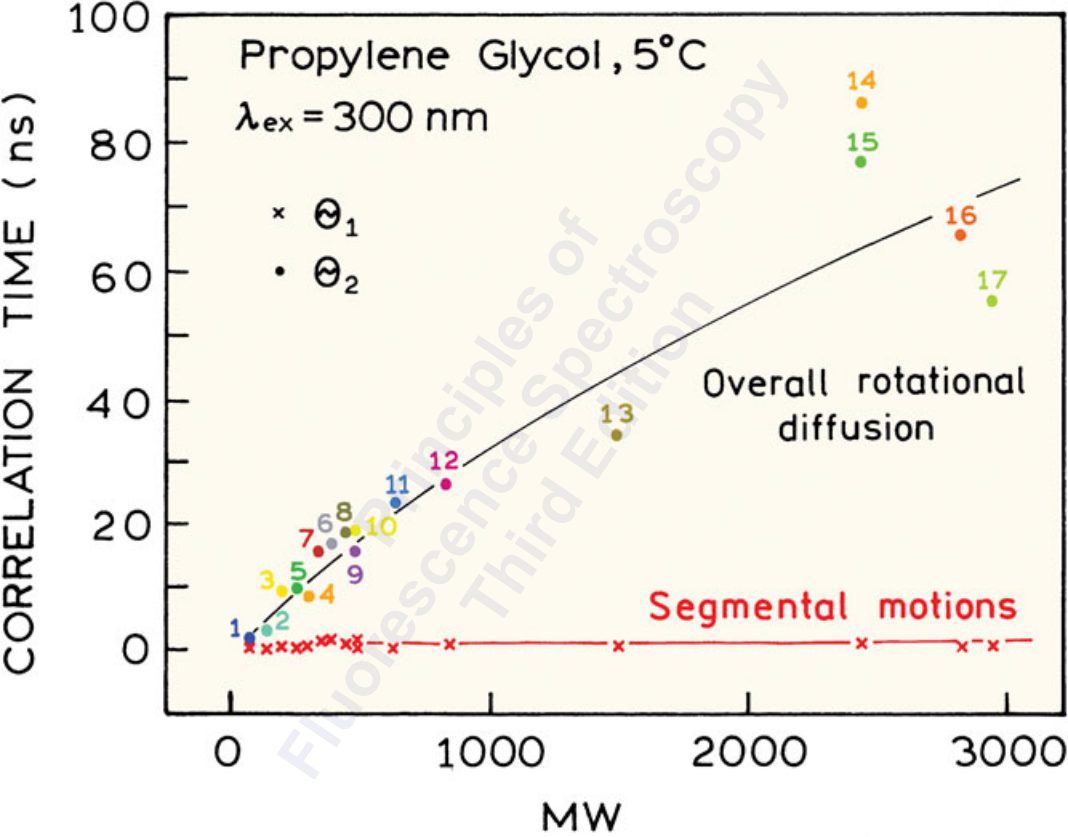


WAVELENGTH (nm)

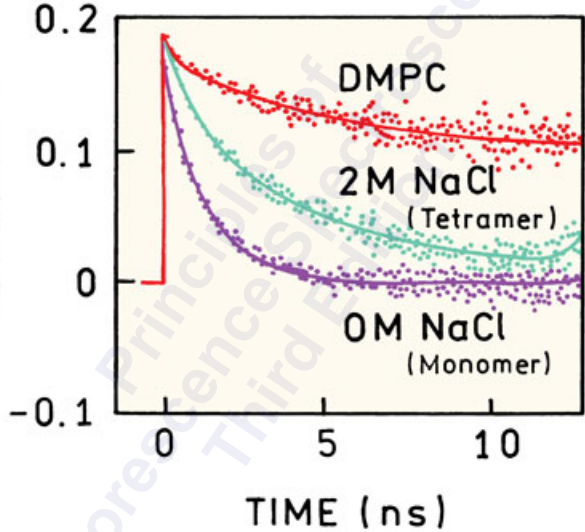




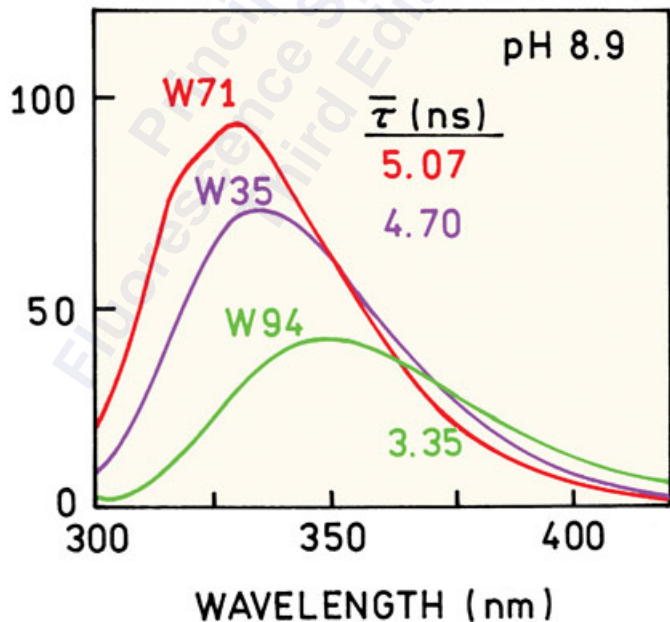
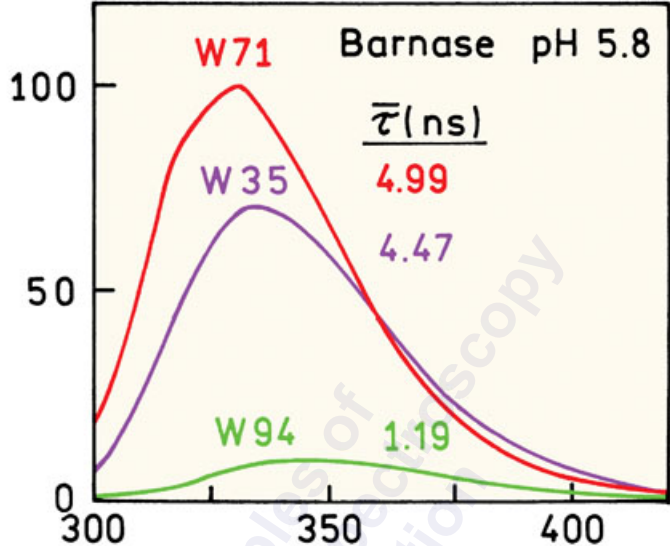


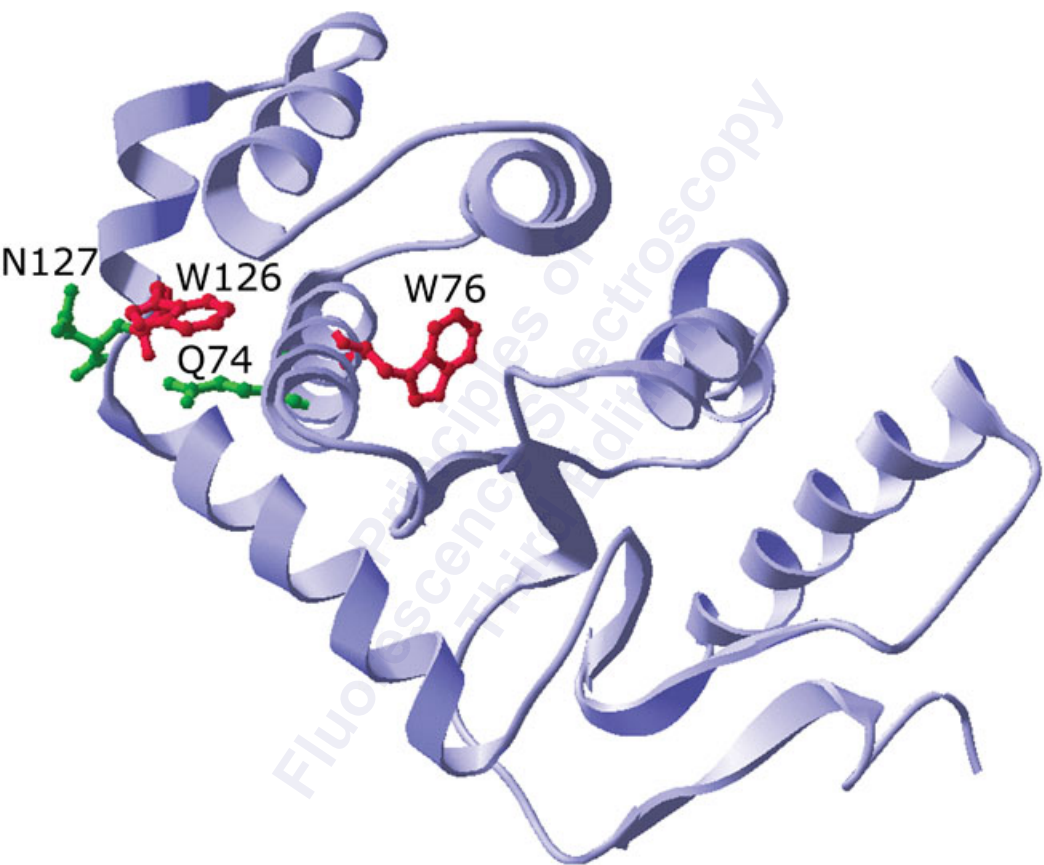


ANISOTROPY

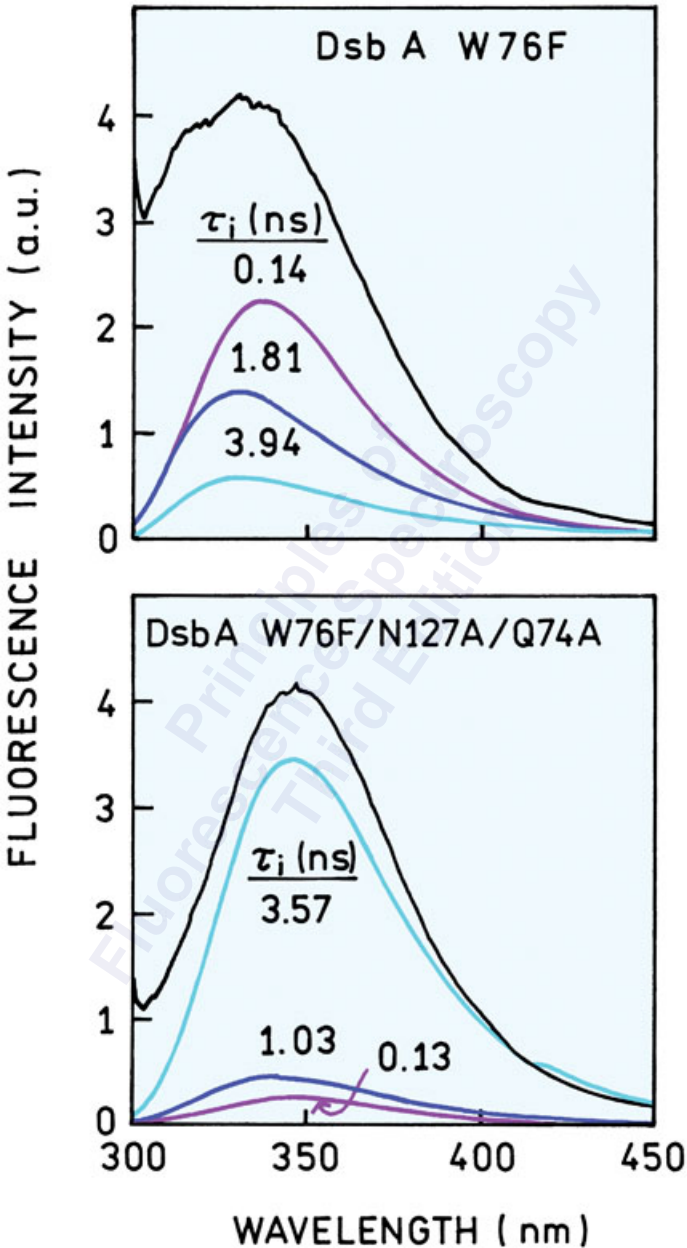


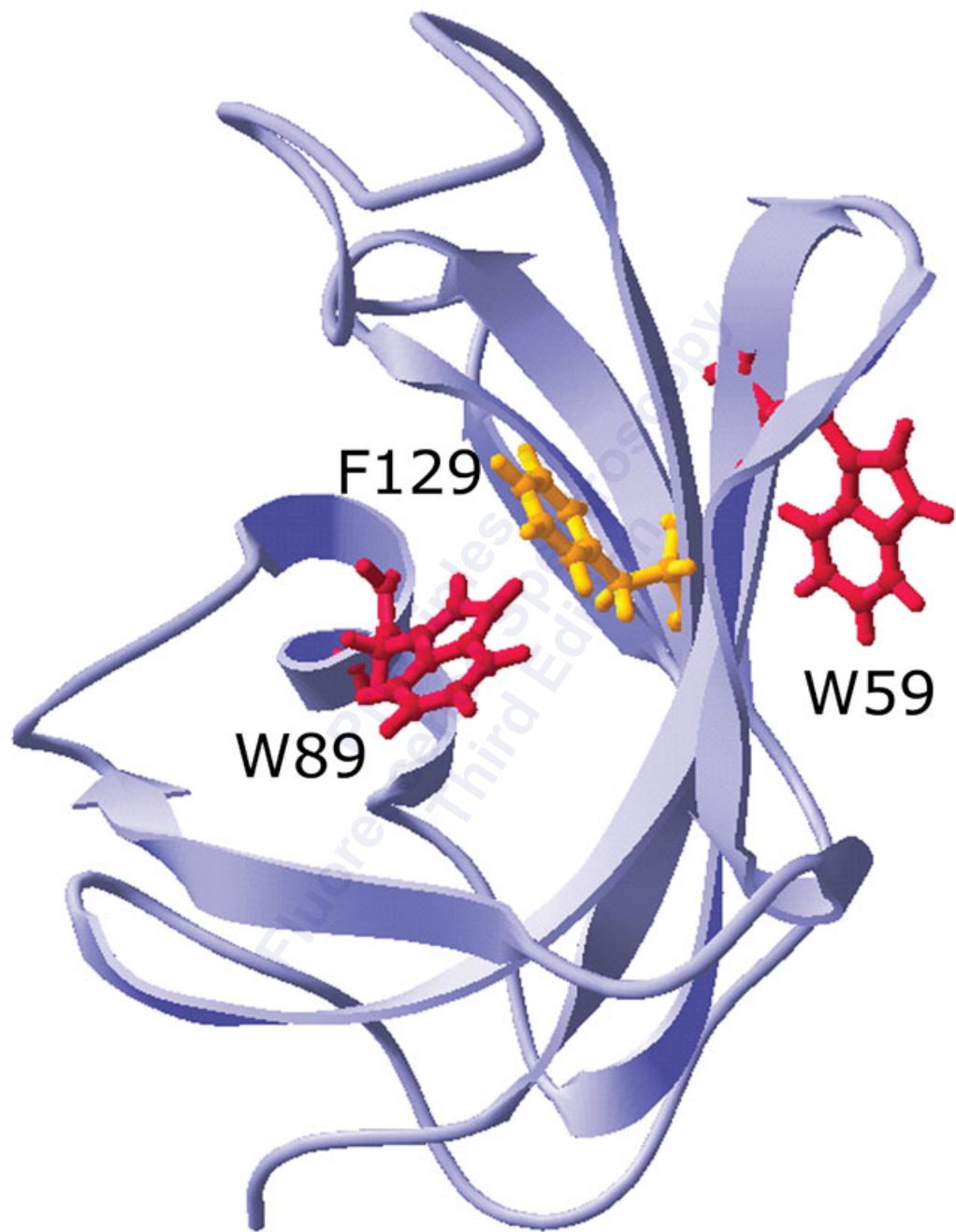
FLUORESCENCE INTENSITY

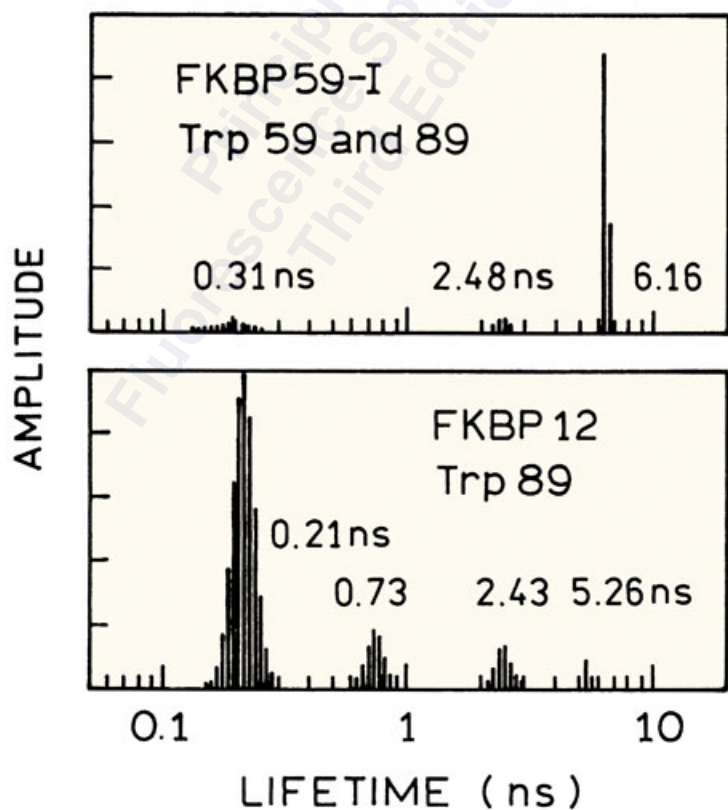
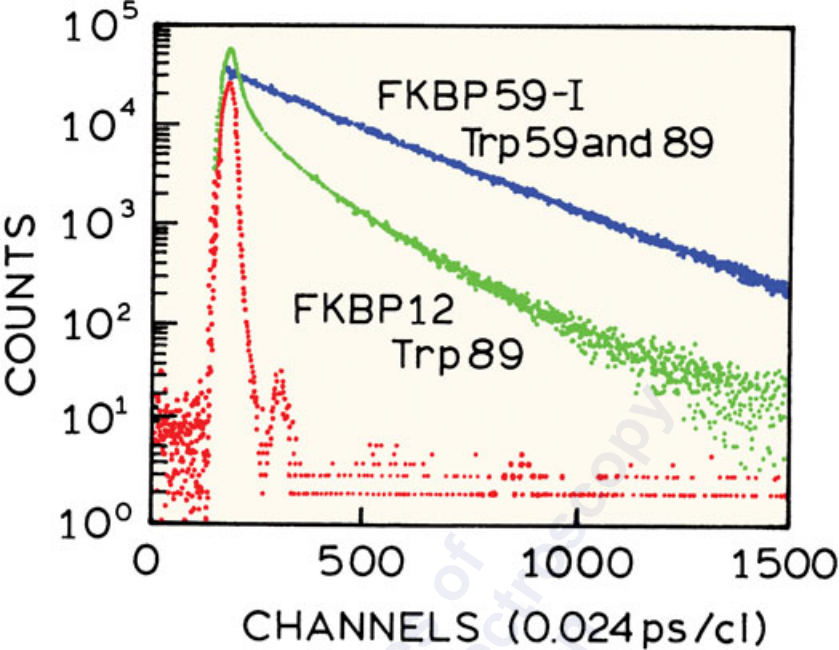


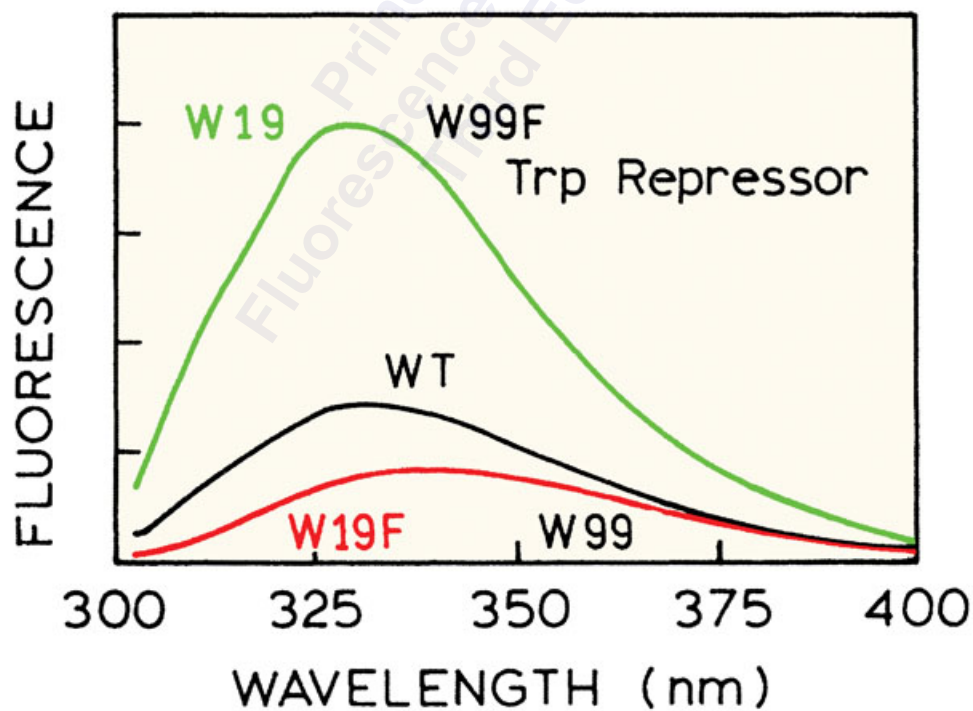
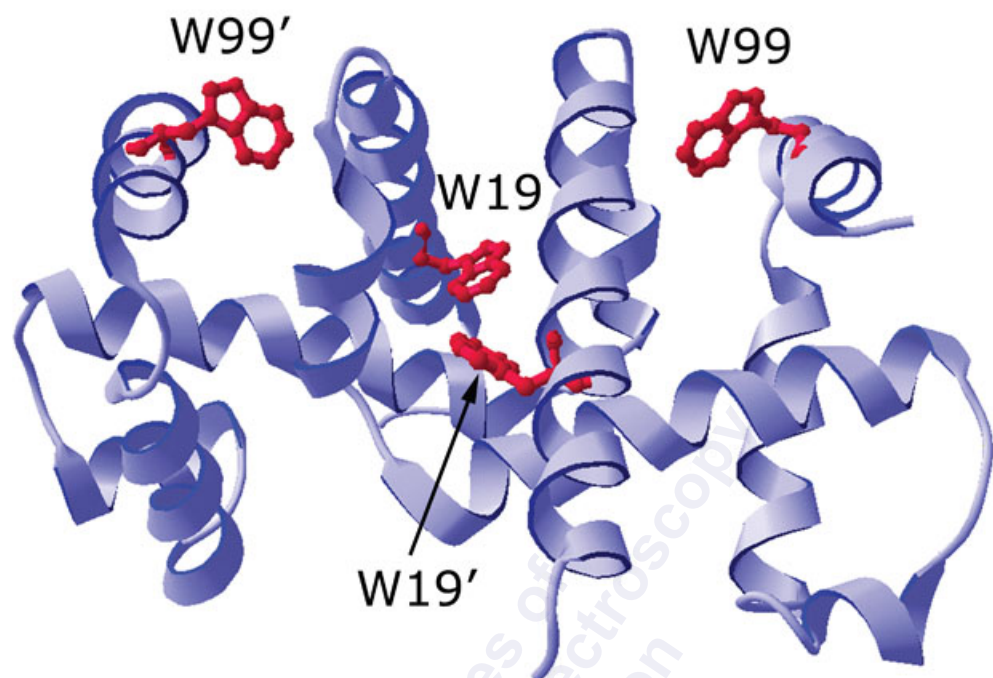


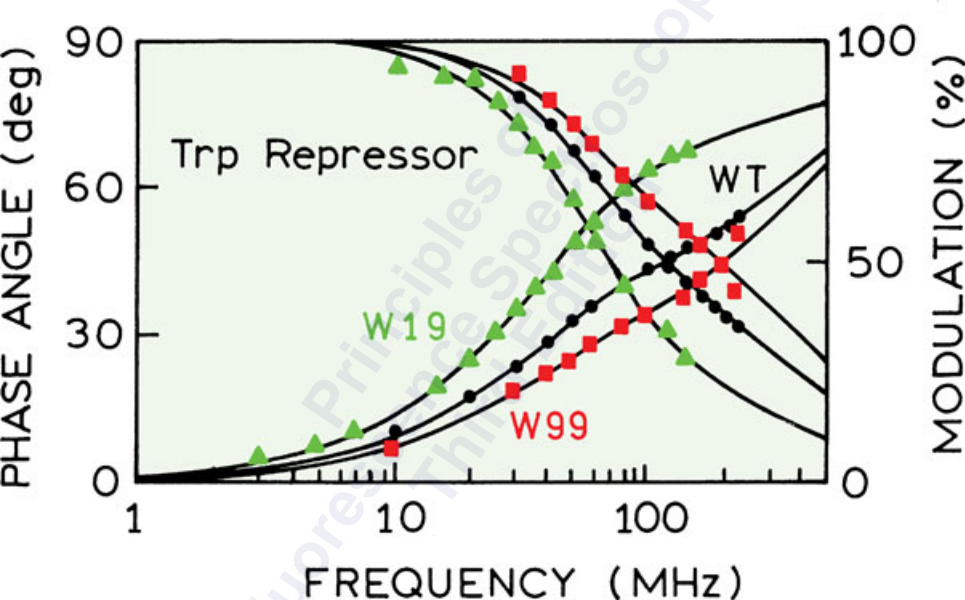




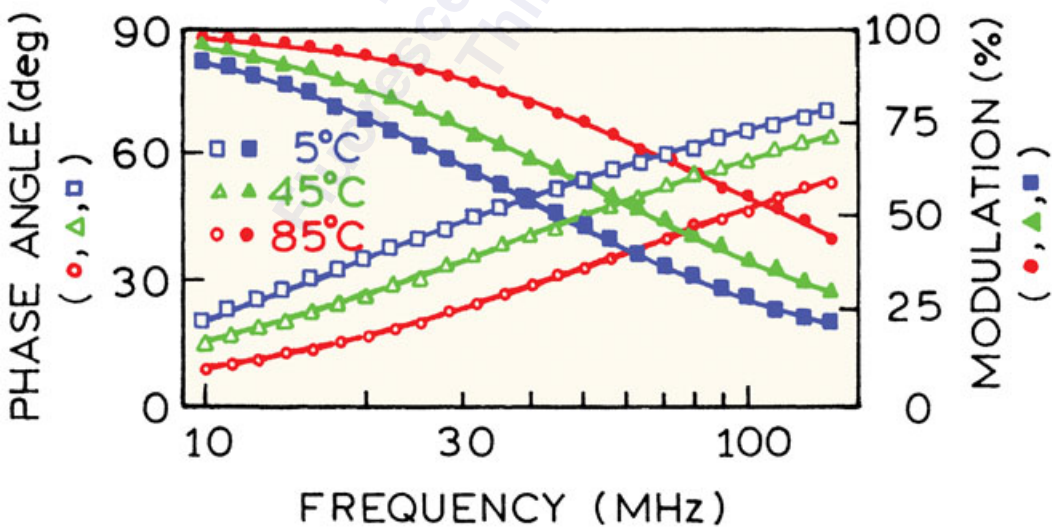
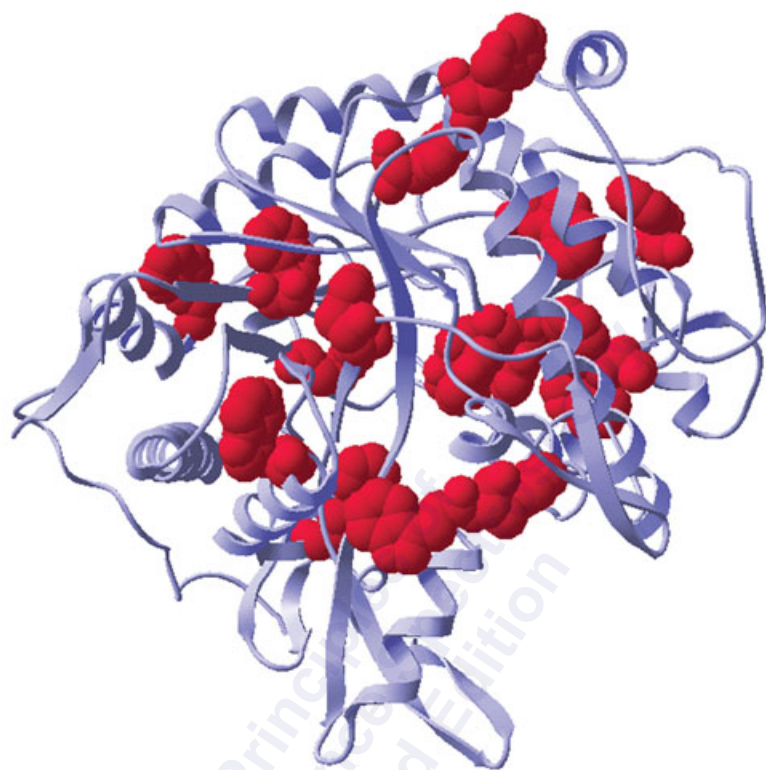




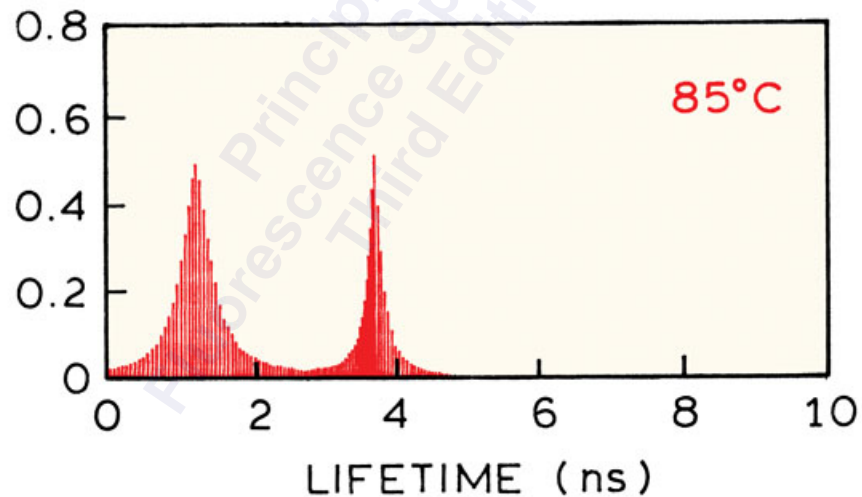
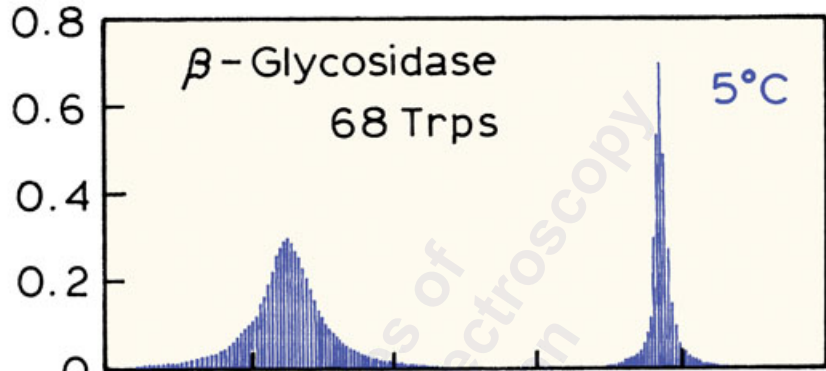


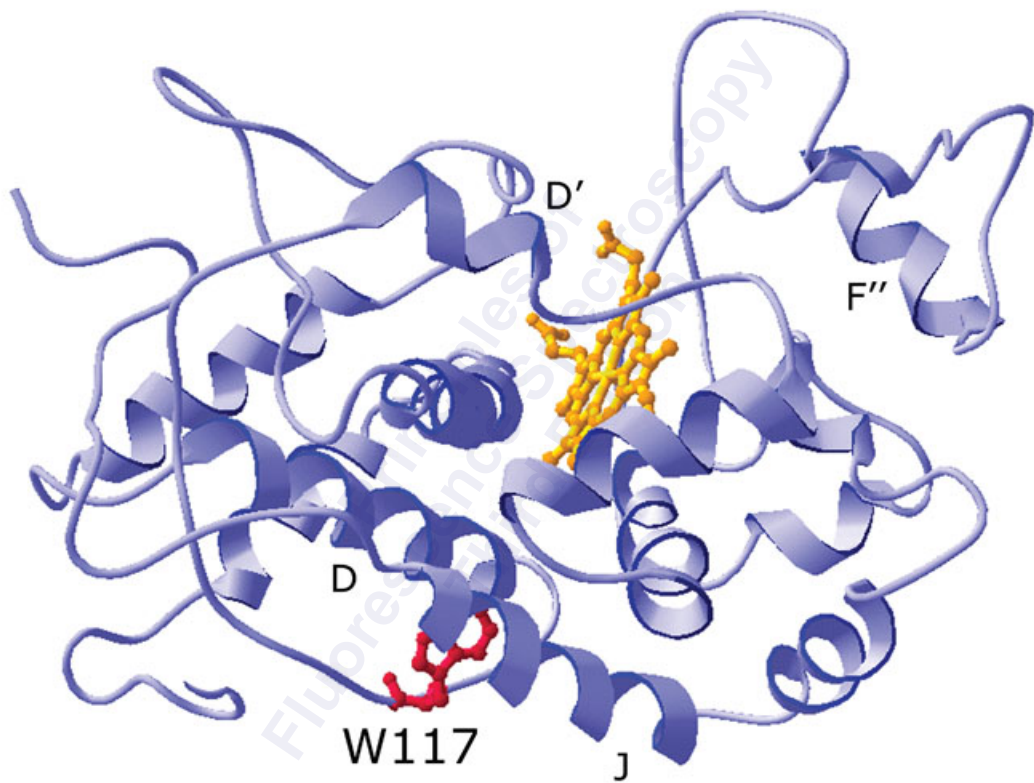




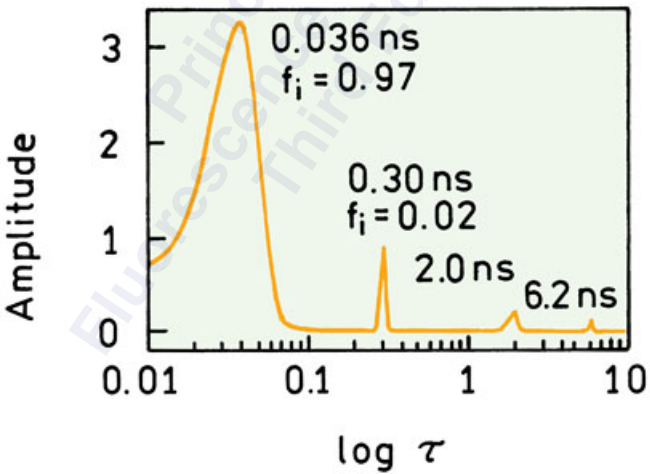
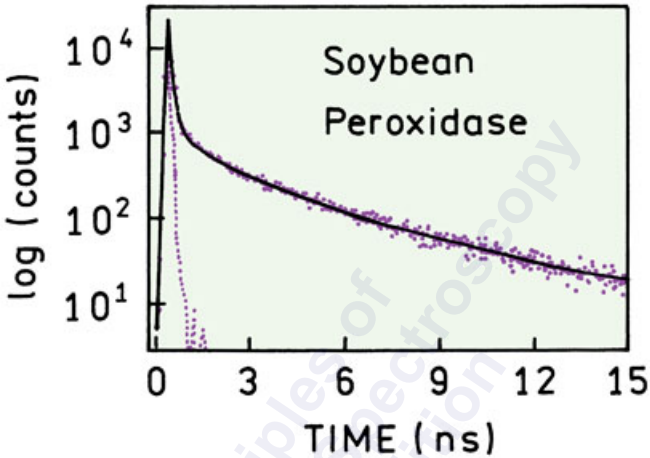


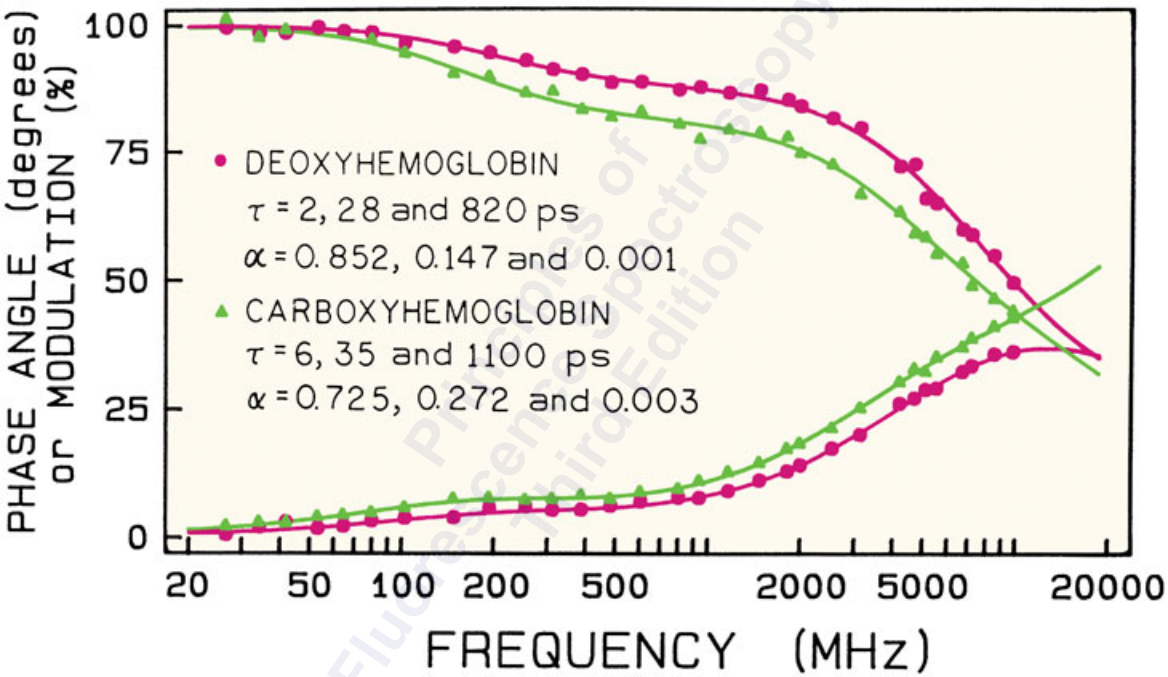
RELATIVE AMPLITUDE

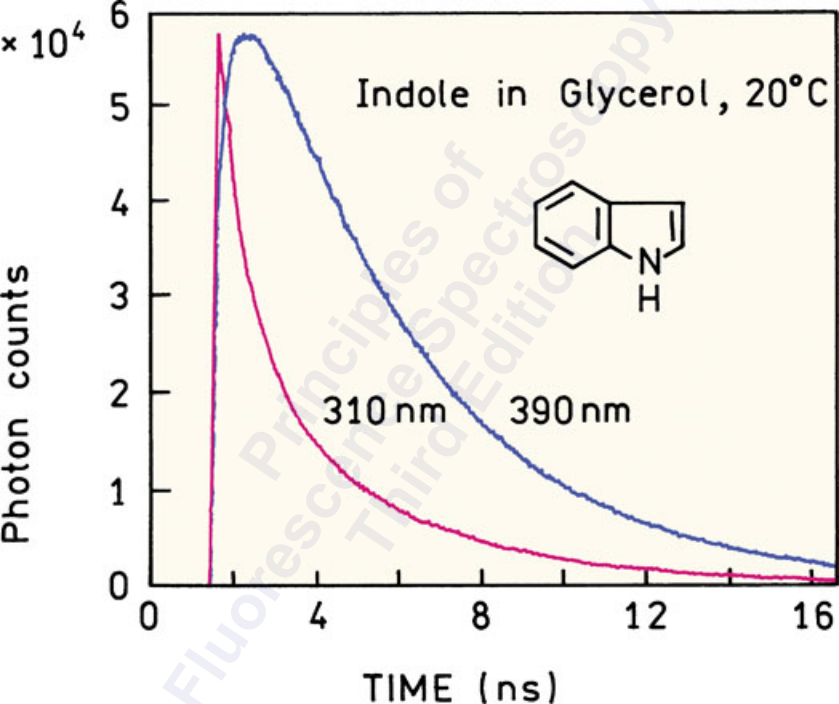


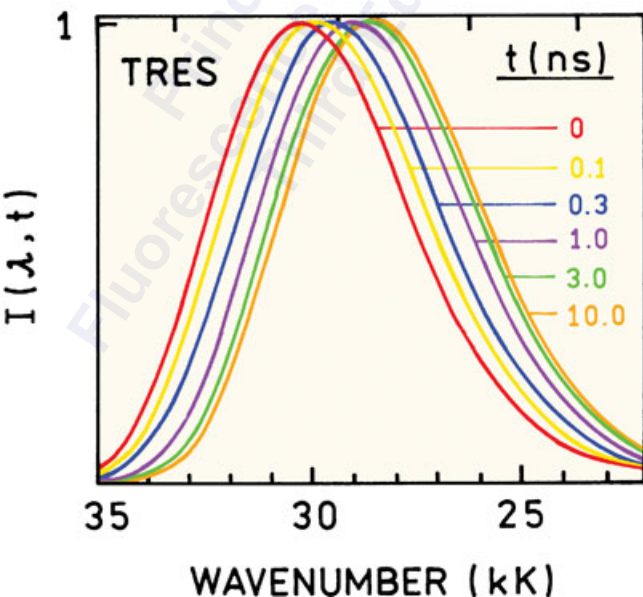
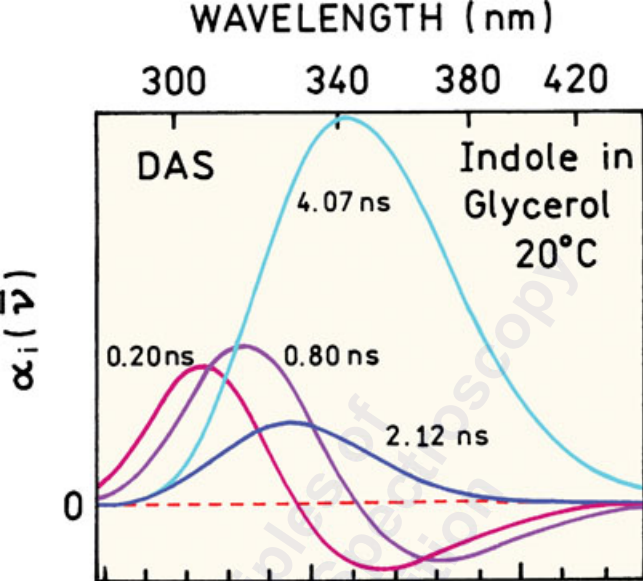


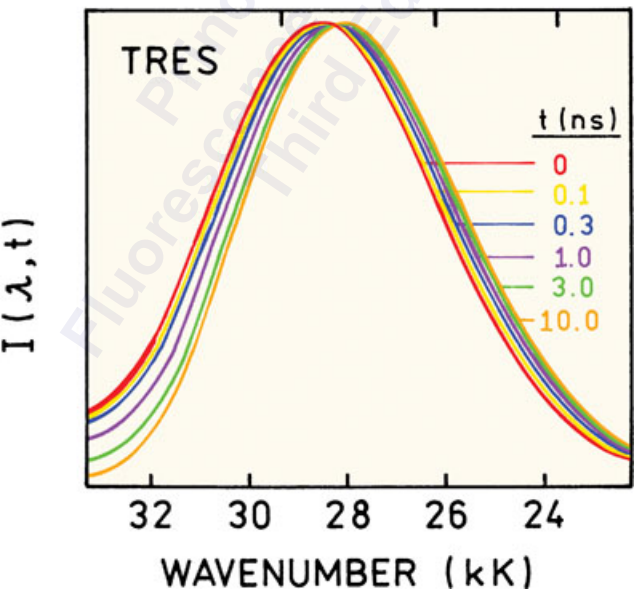
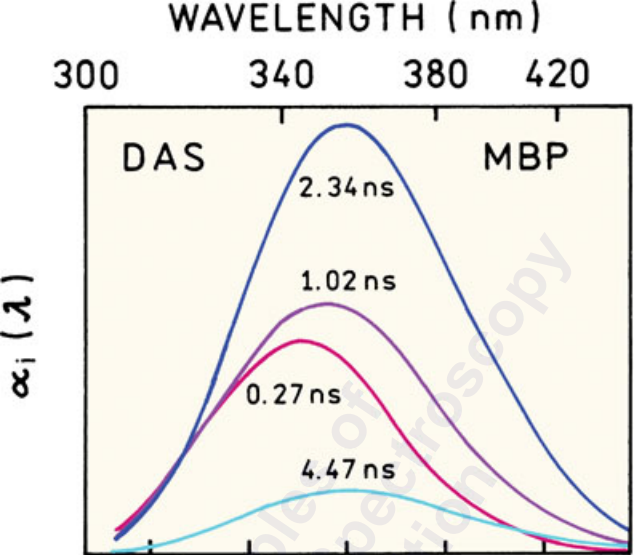


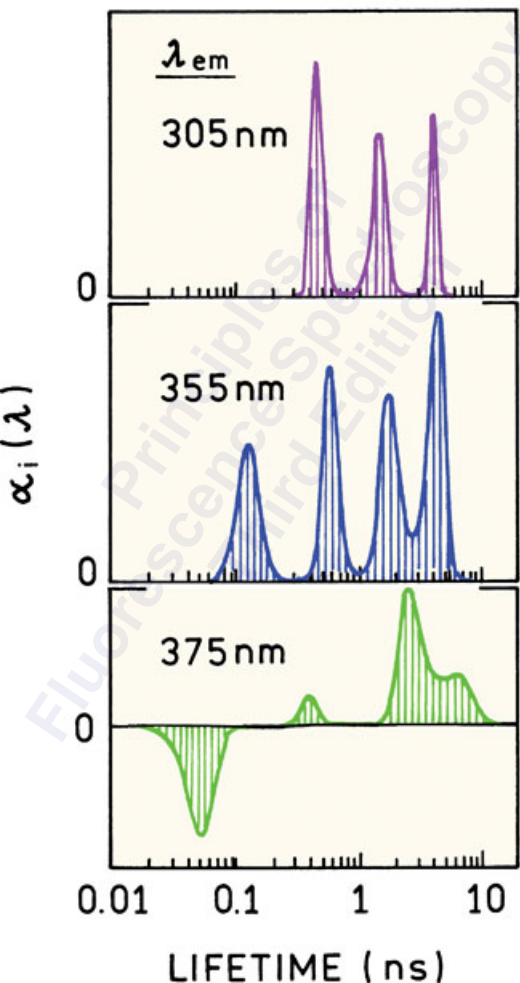
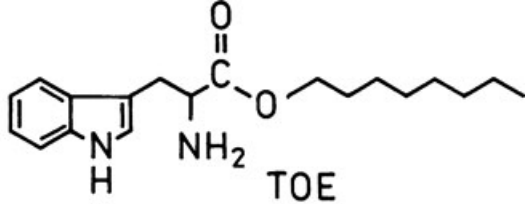


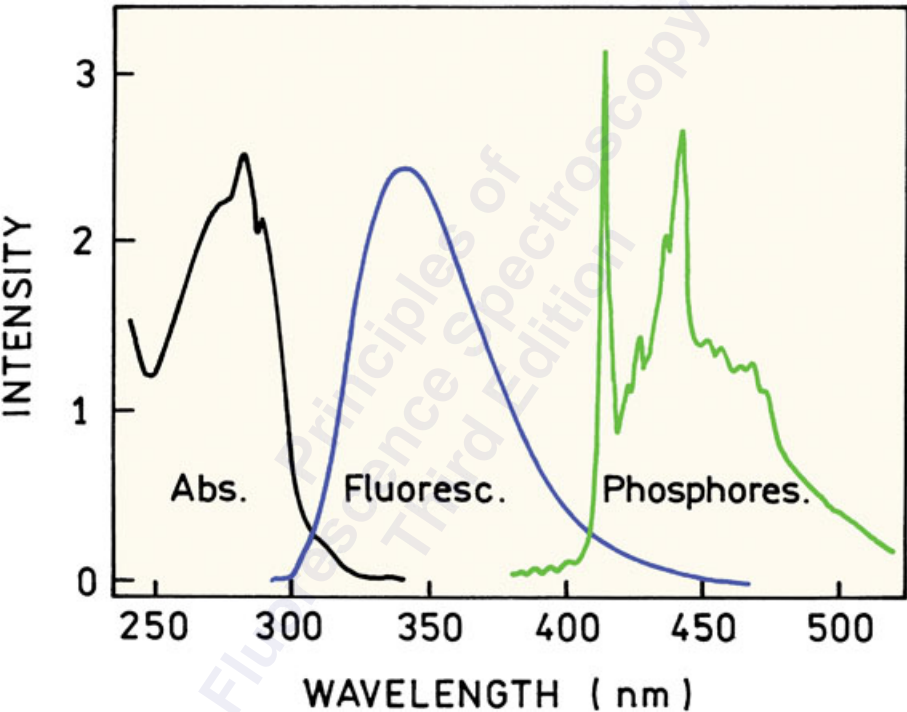




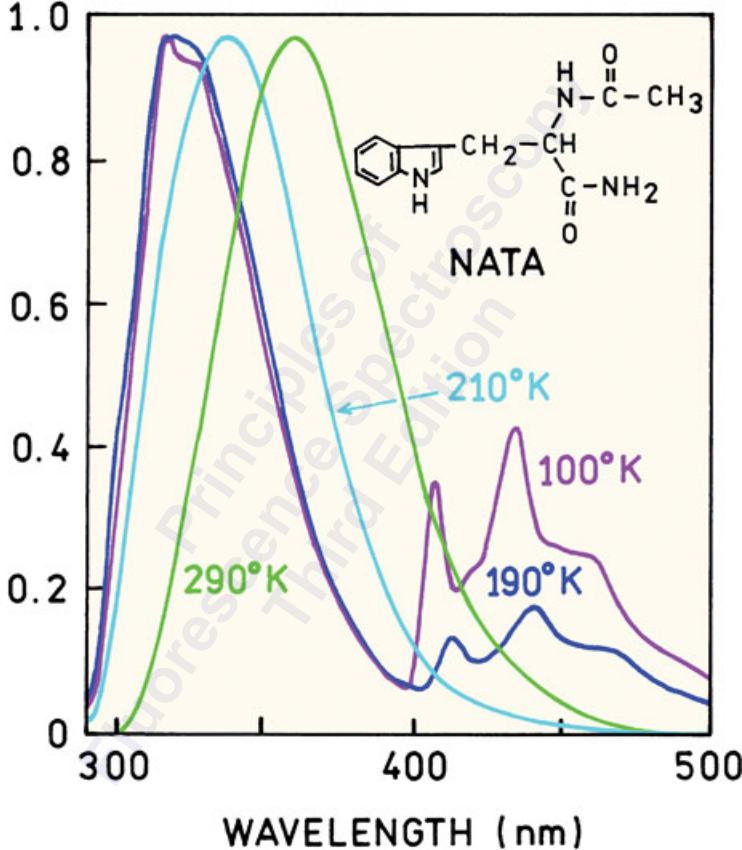




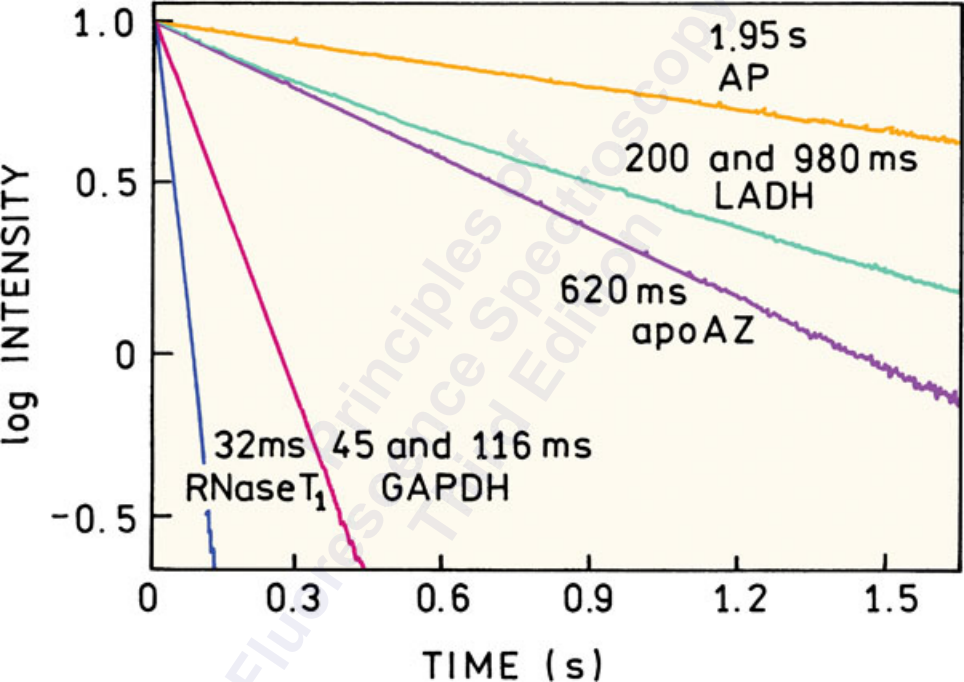


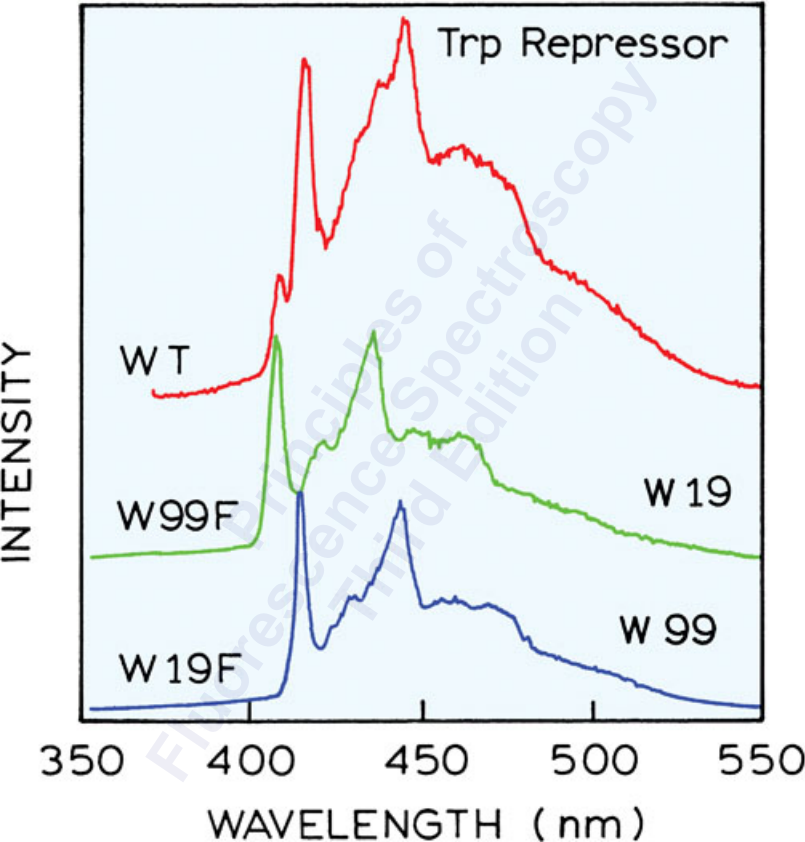


NORMALIZED INTENSITY

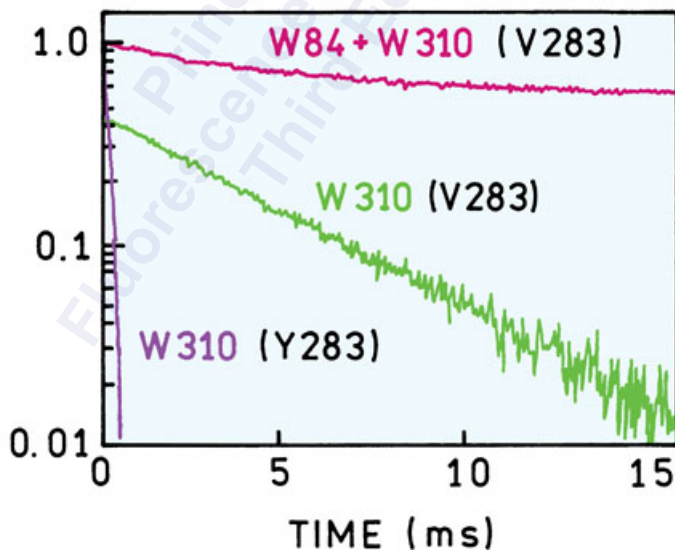
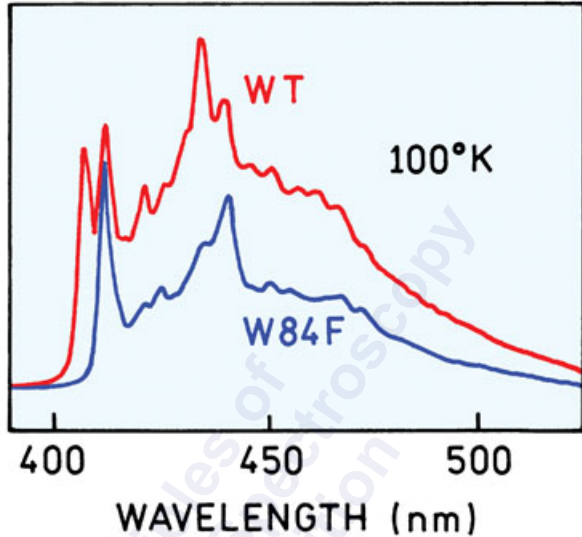


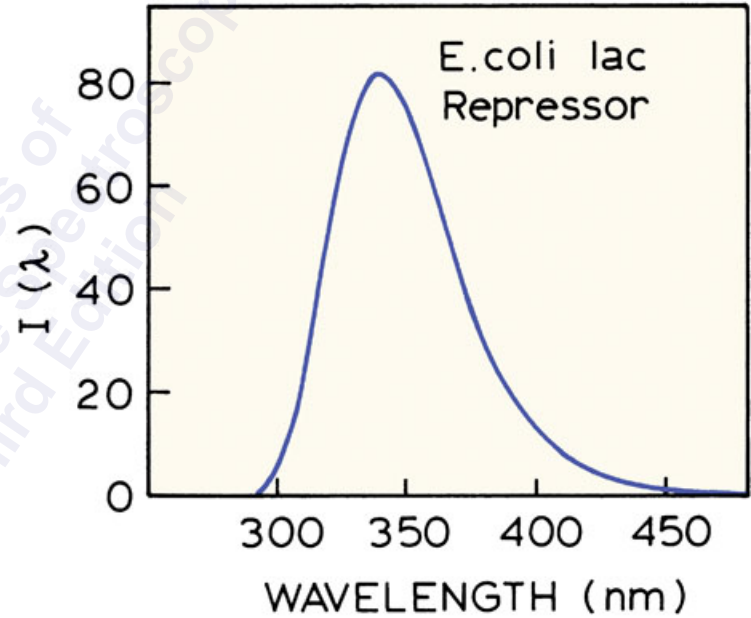
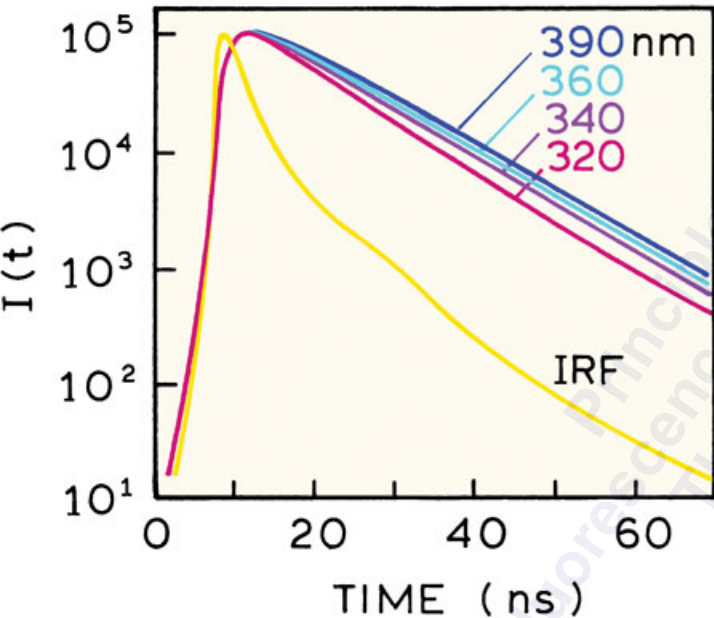


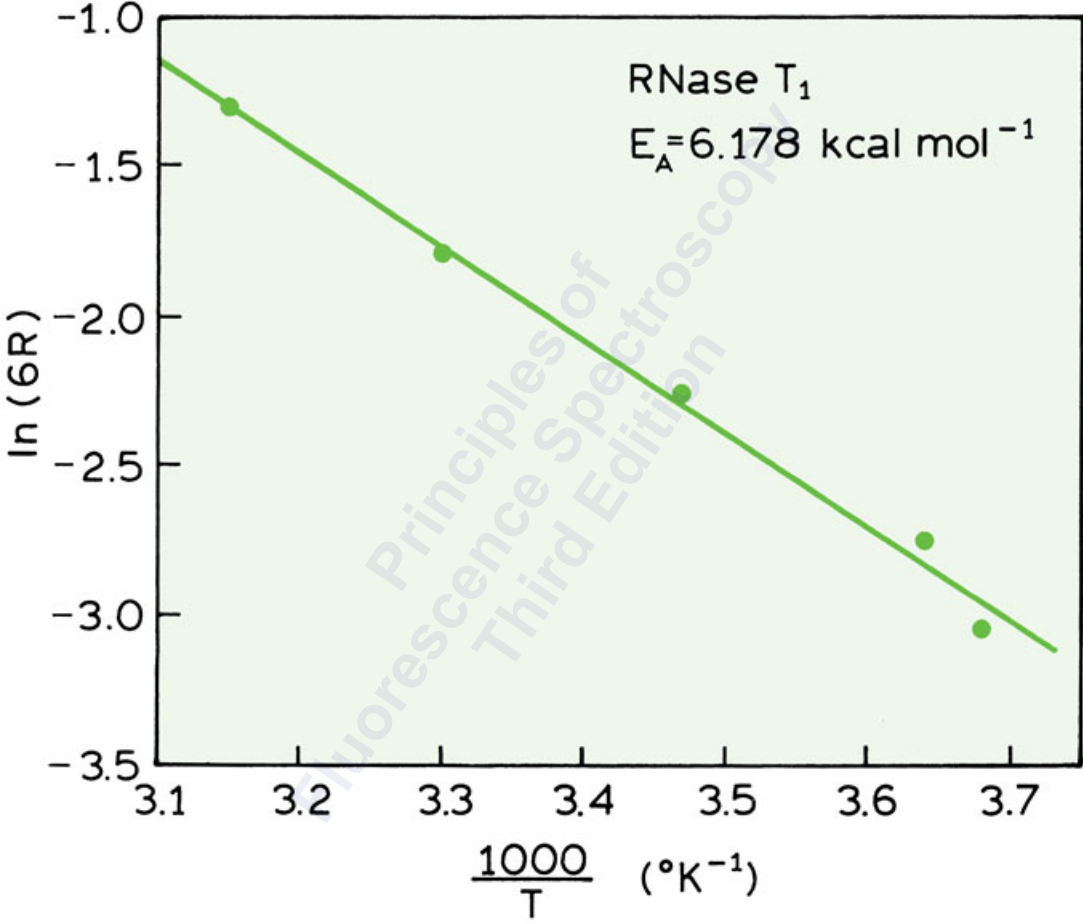




PHOSPHORESCENCE INTENSITY







INTENSITY

80

60

40

20

0

9.8 ns

3.8 ns

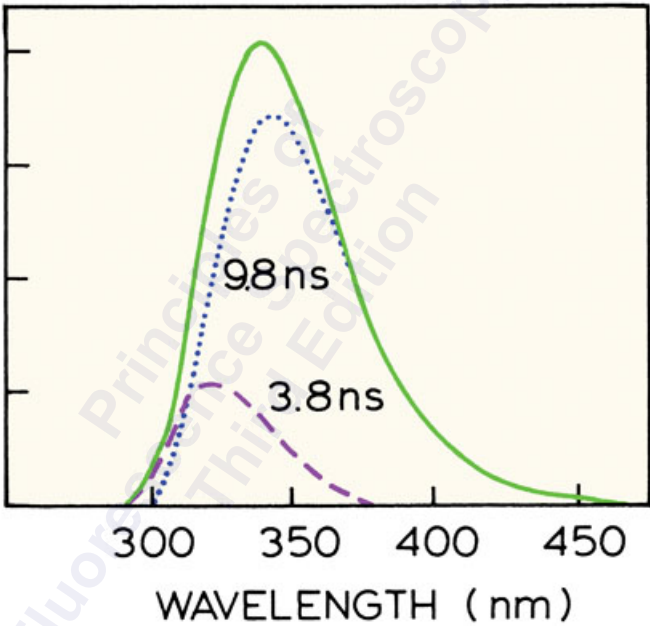
300

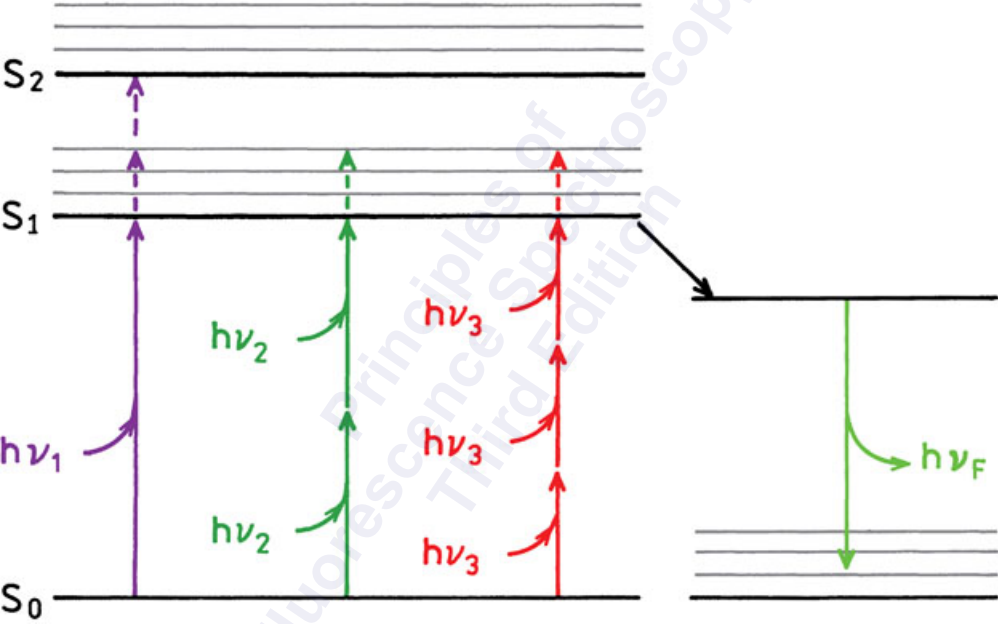
350

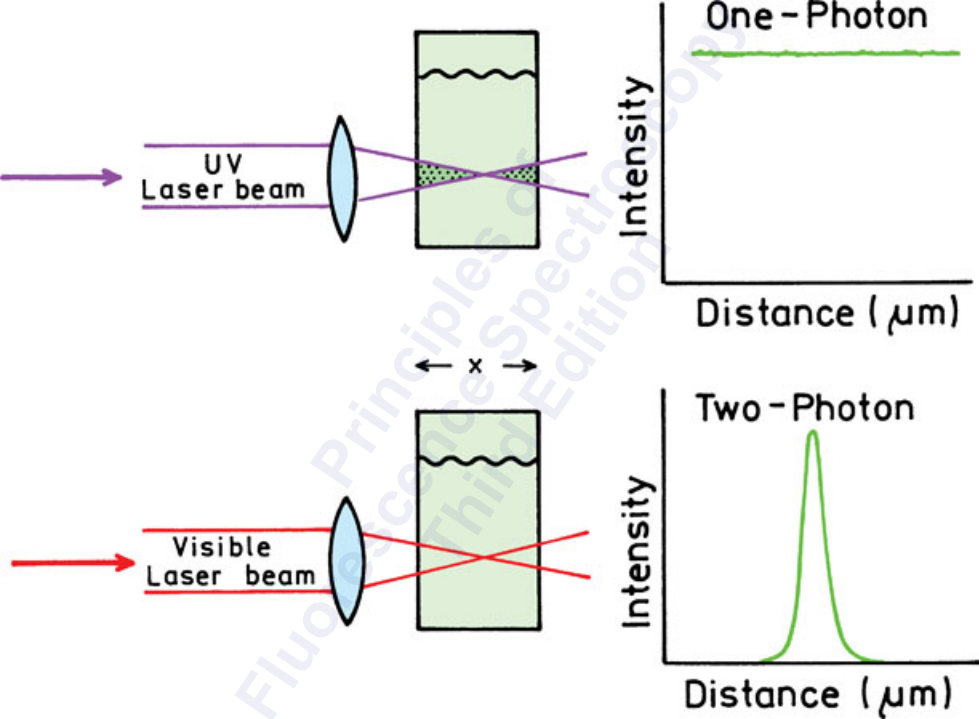
400

450

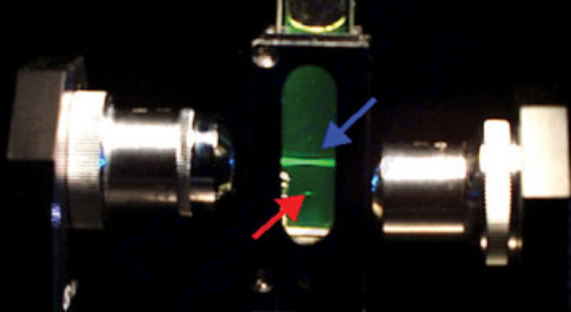
WAVELENGTH (nm)

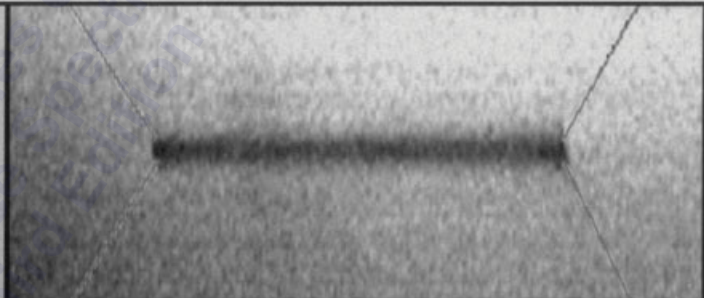
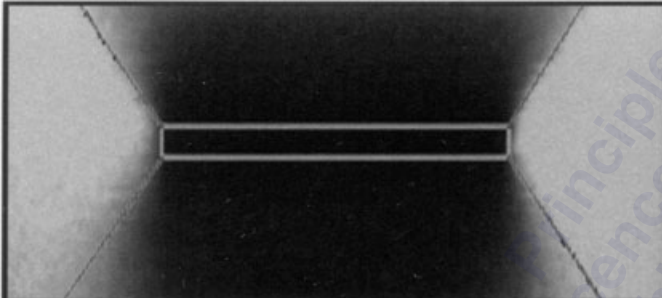


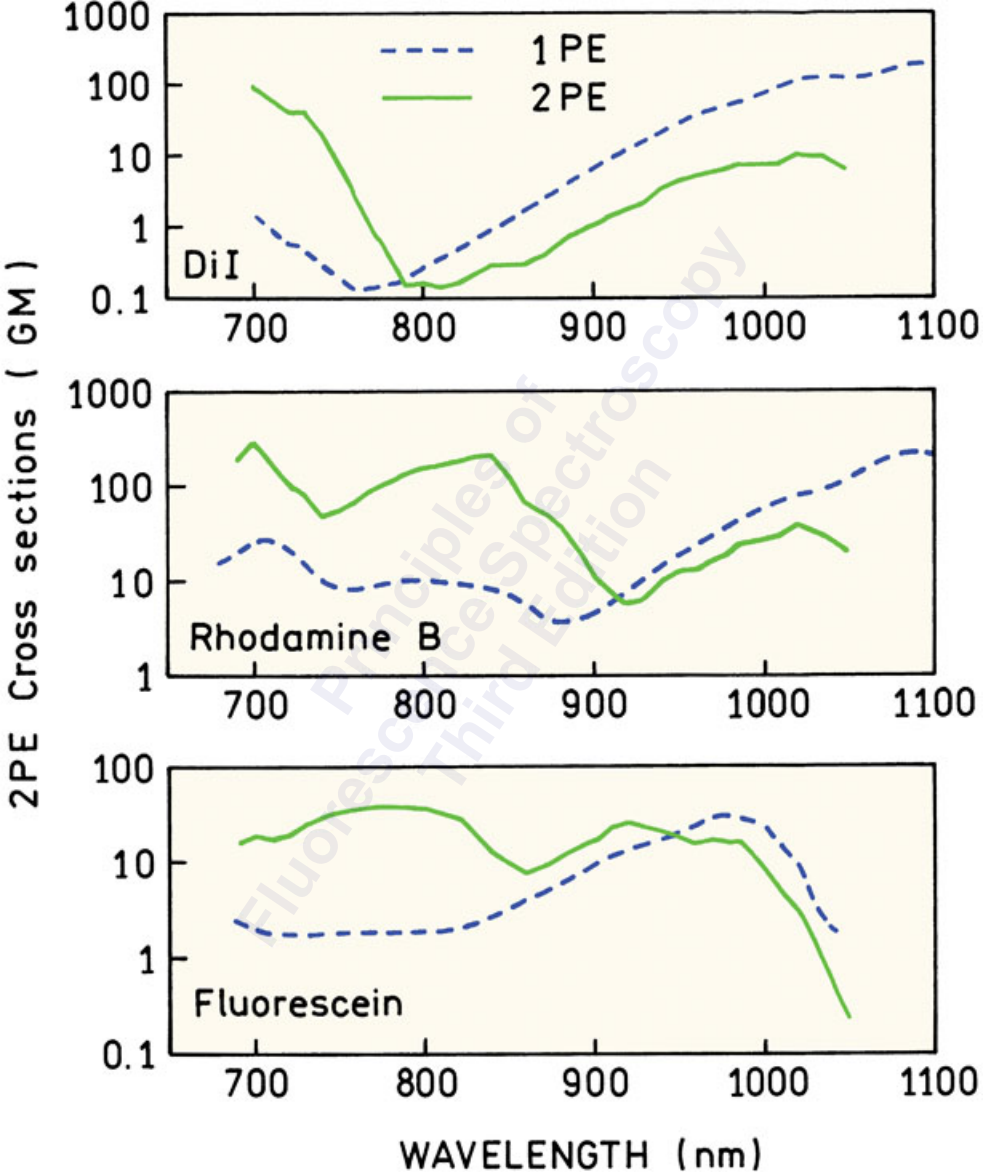


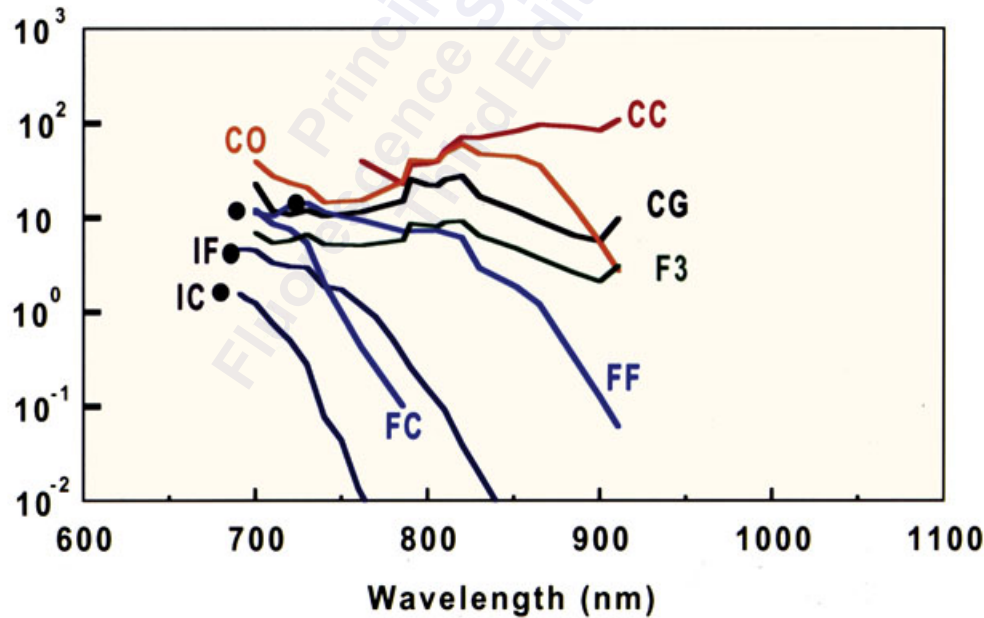
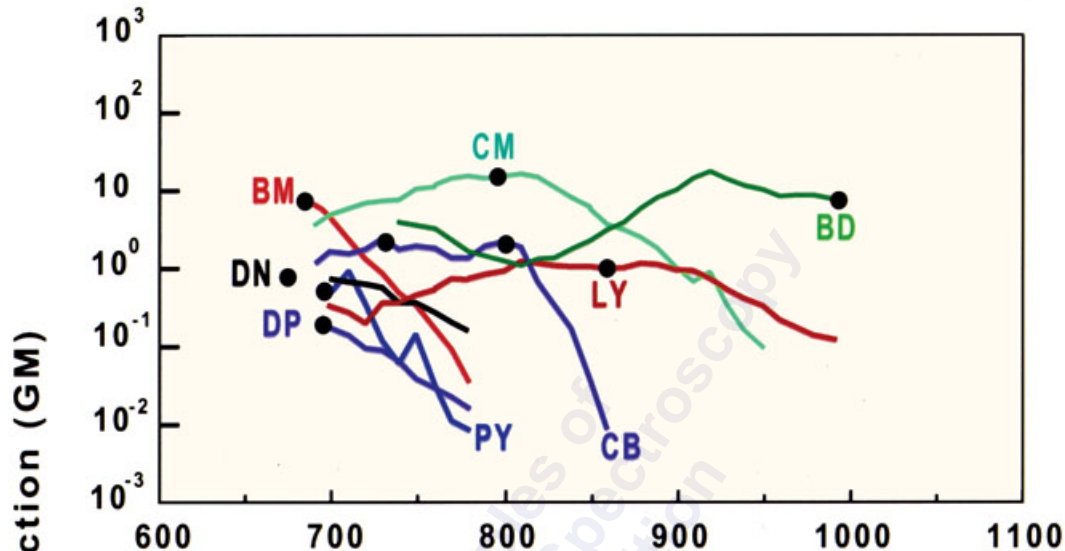












Sulfo-  
rhodamine B

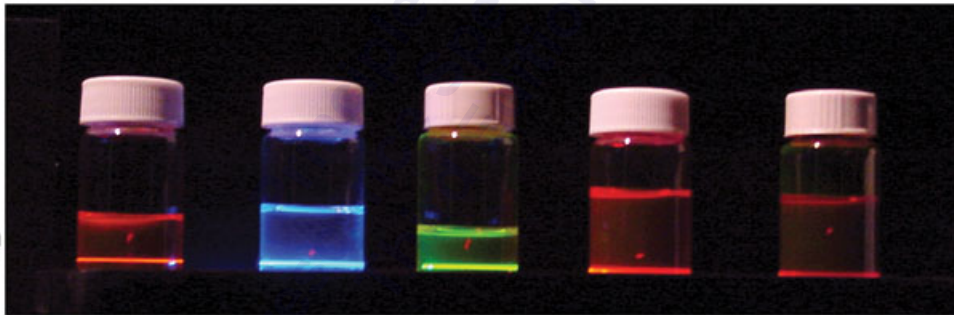
Coumarin  
102

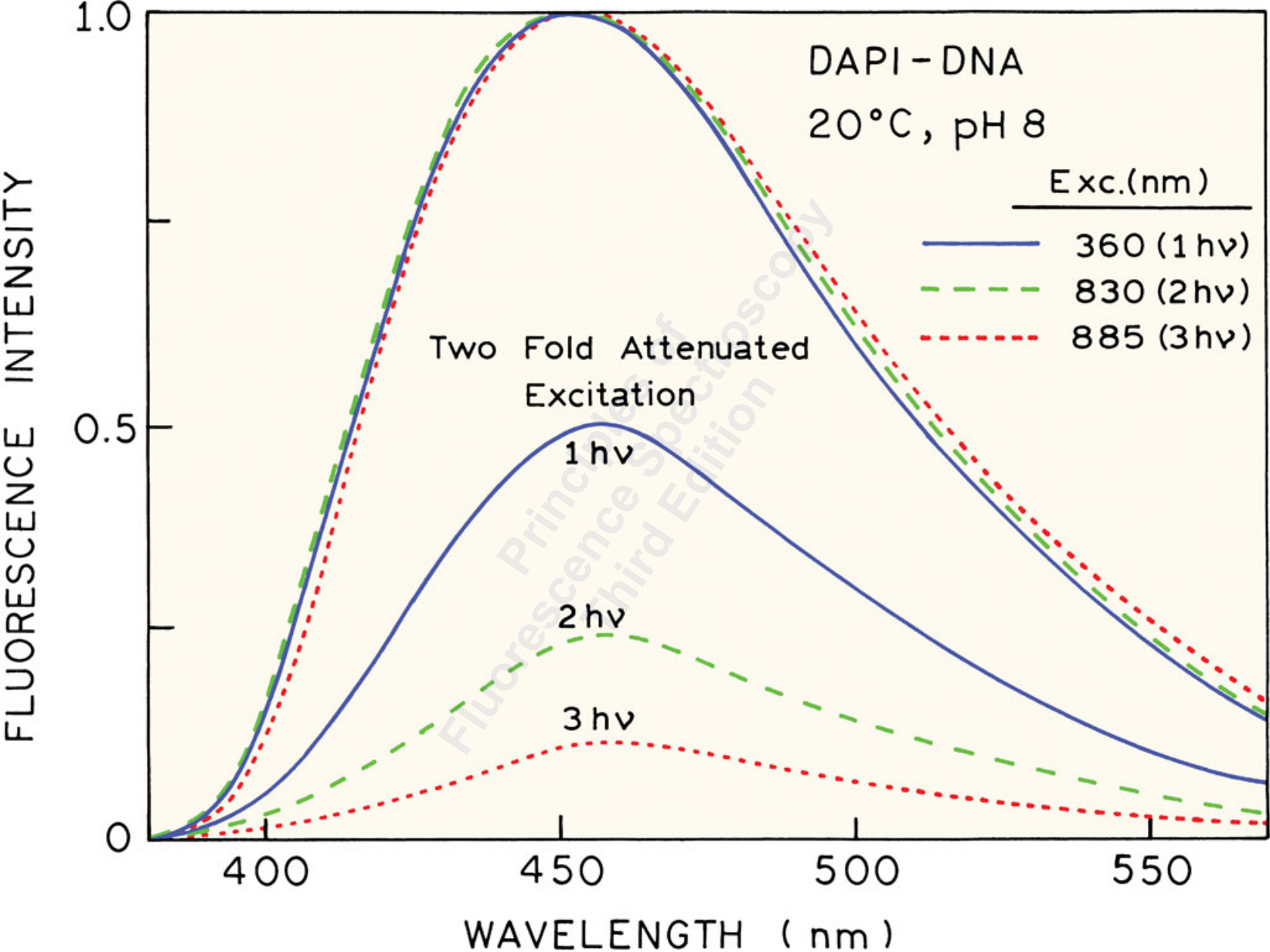
Fluorescein

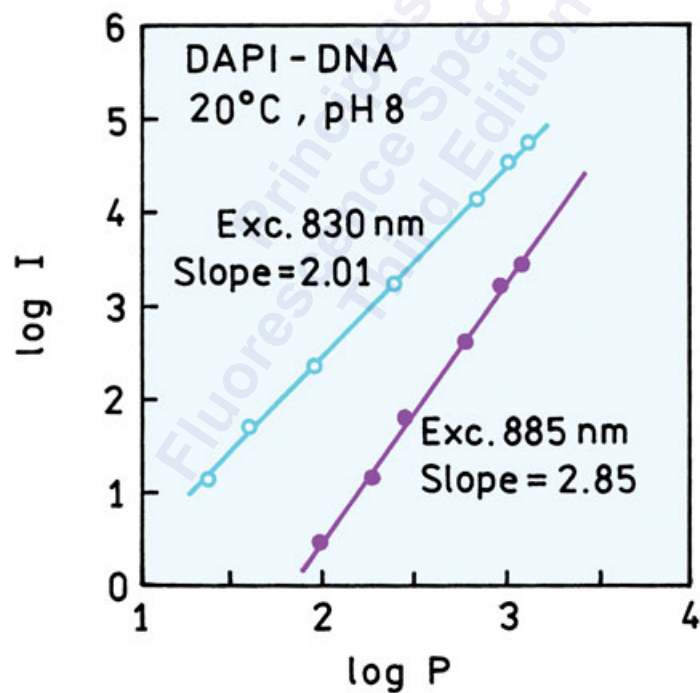
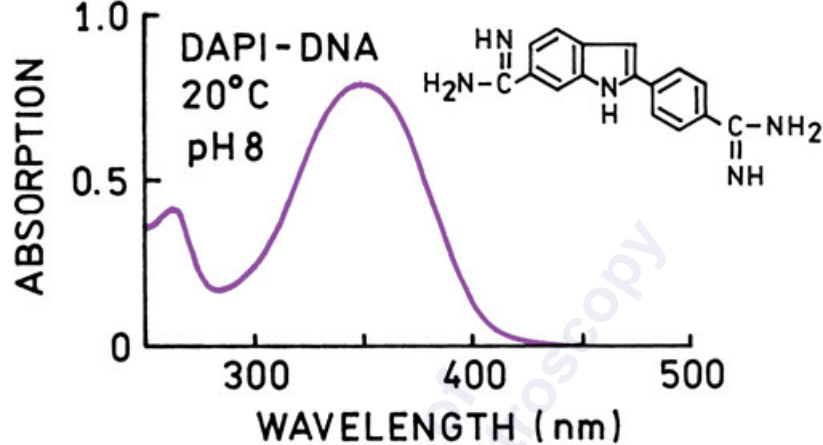
Rhodamine B

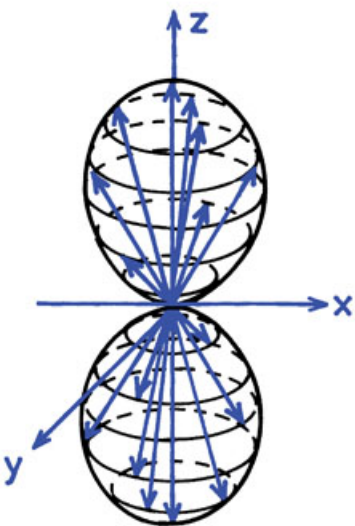
$[\text{Ru}(\text{bpy})_3]^{2+}$

800 nm



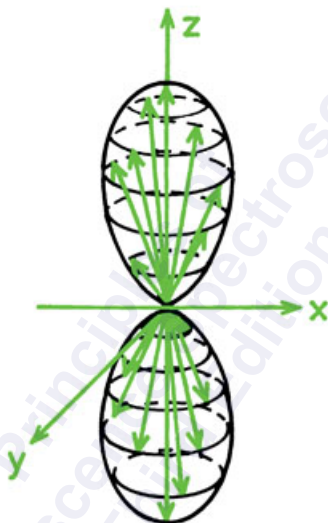






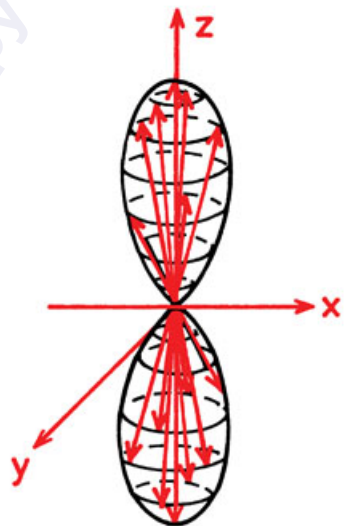
1  $h\nu$

$$r_{01} = 2/5$$



2  $h\nu$

$$r_{02} = 4/7$$

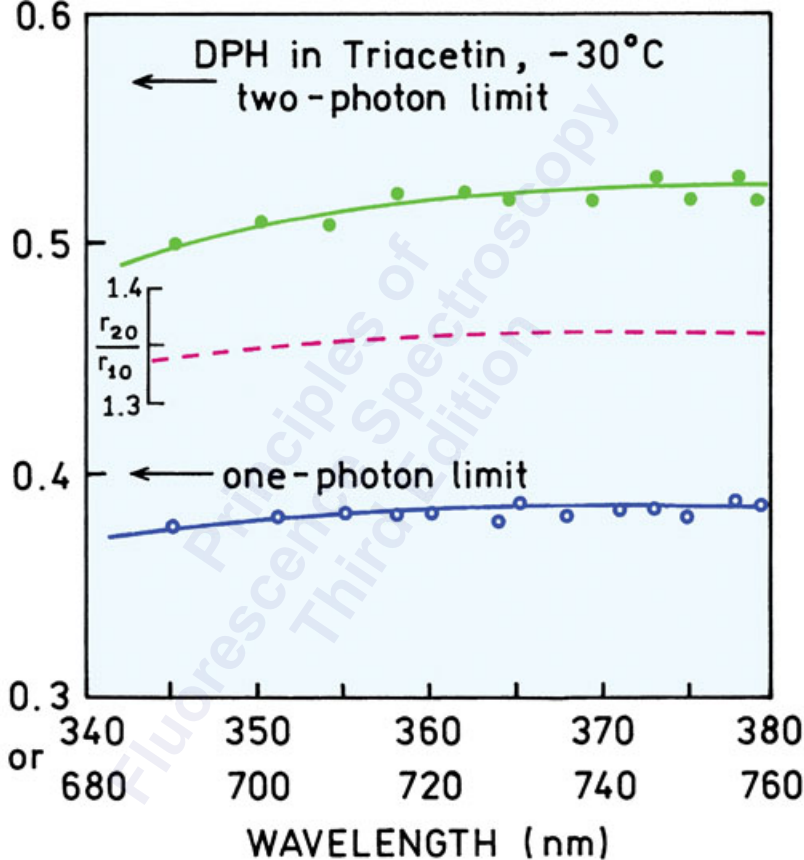


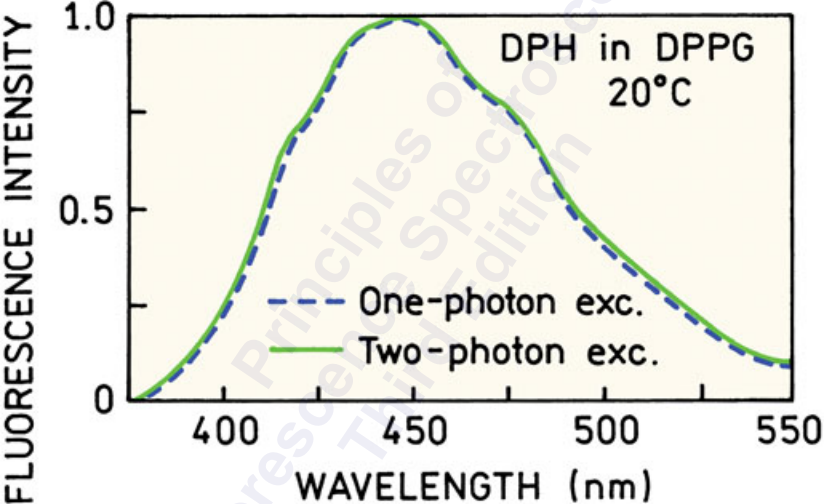
3  $h\nu$

$$r_{03} = 2/3$$

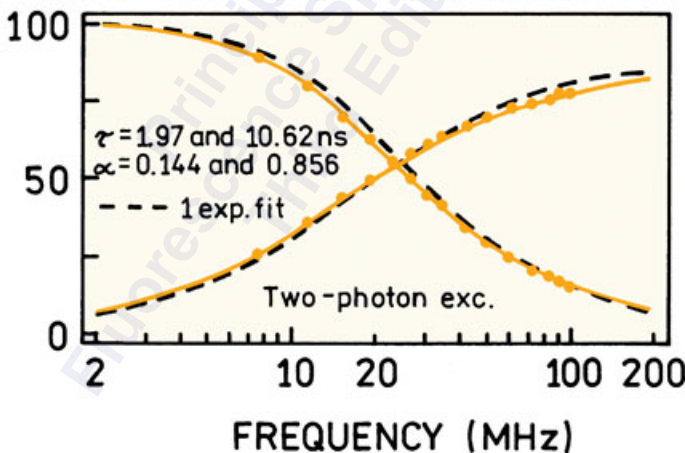
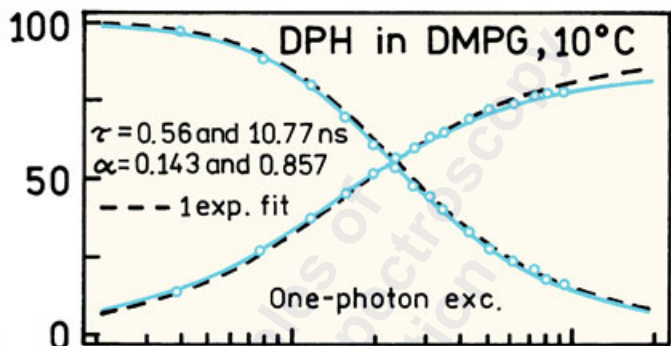


ANISOTROPY





PHASE ANGLE (deg) or MODULATION(%)



ANISOTROPY

DPH in DPPG

0.4

0.3

0.2

0.1

0

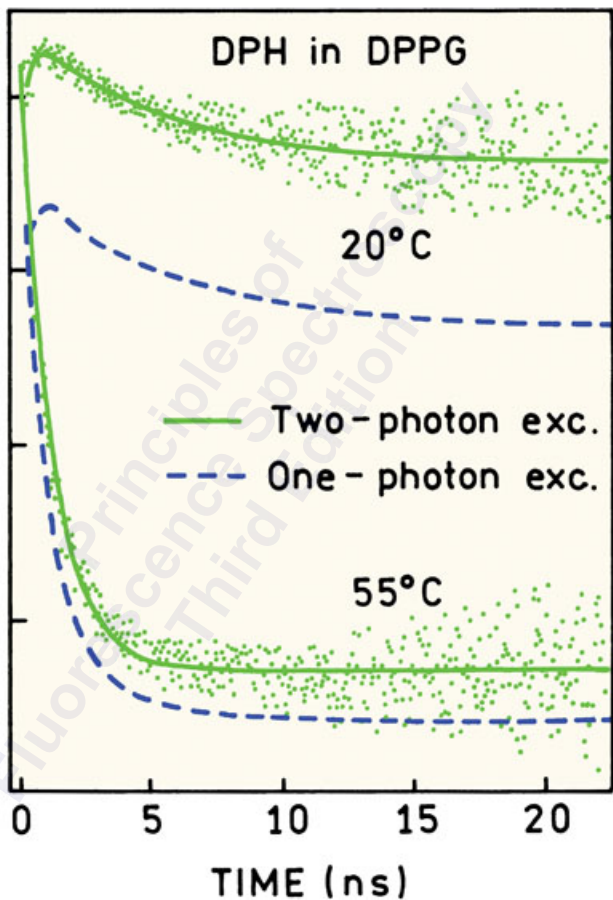
20°C

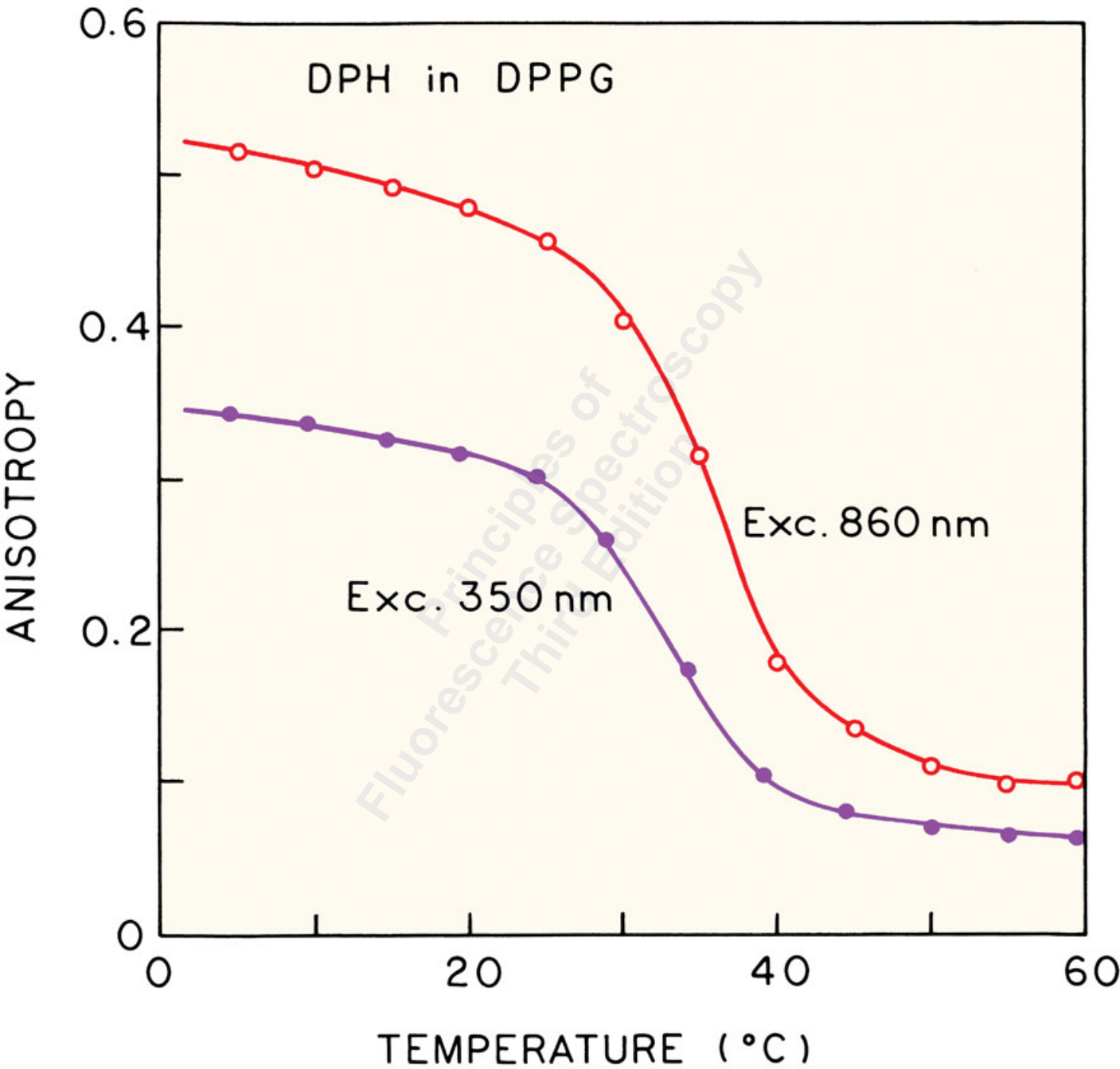
55°C

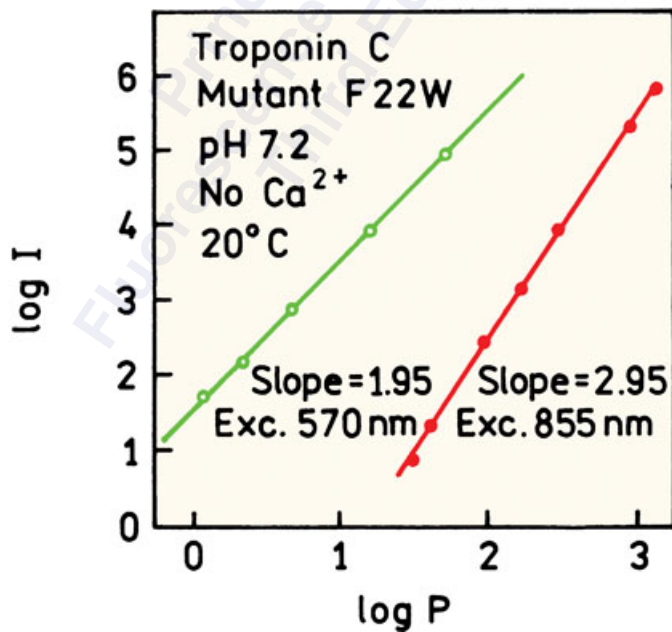
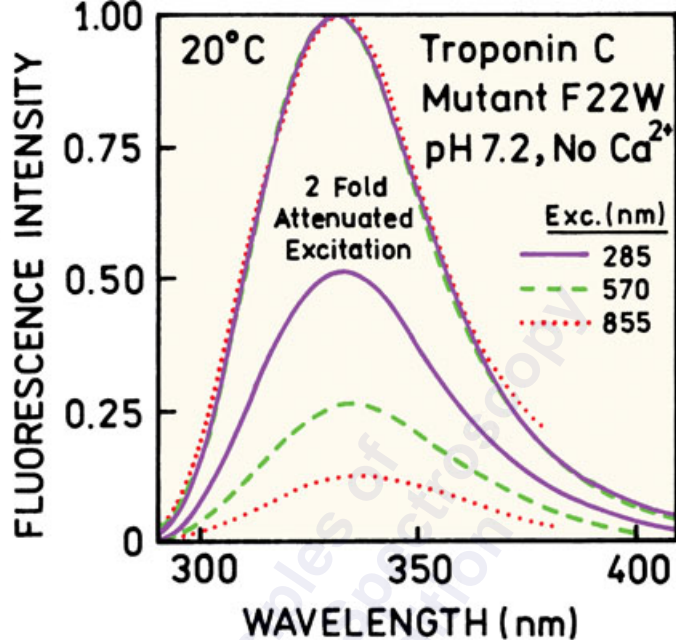
— Two-photon exc.

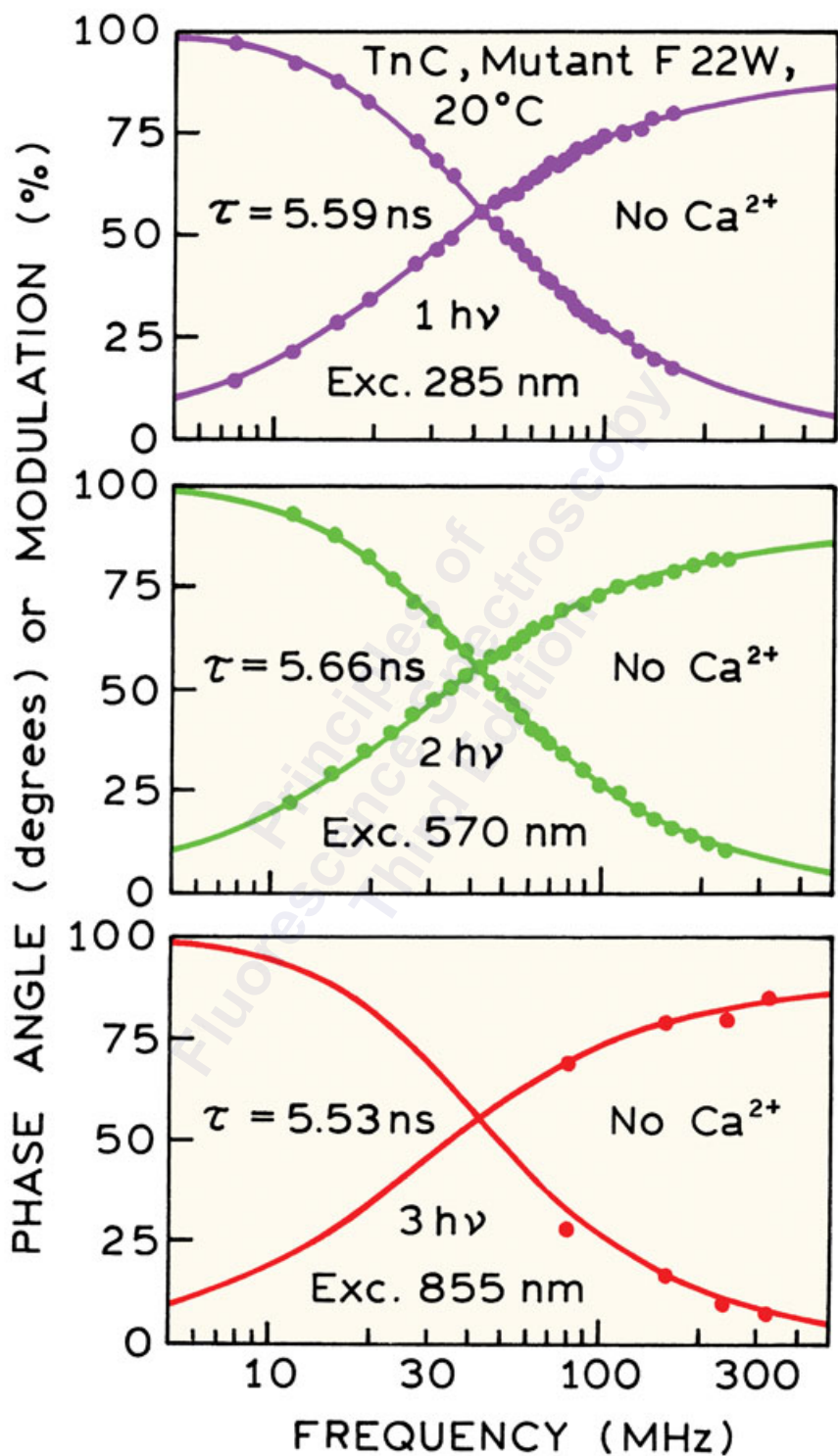
- - - One-photon exc.

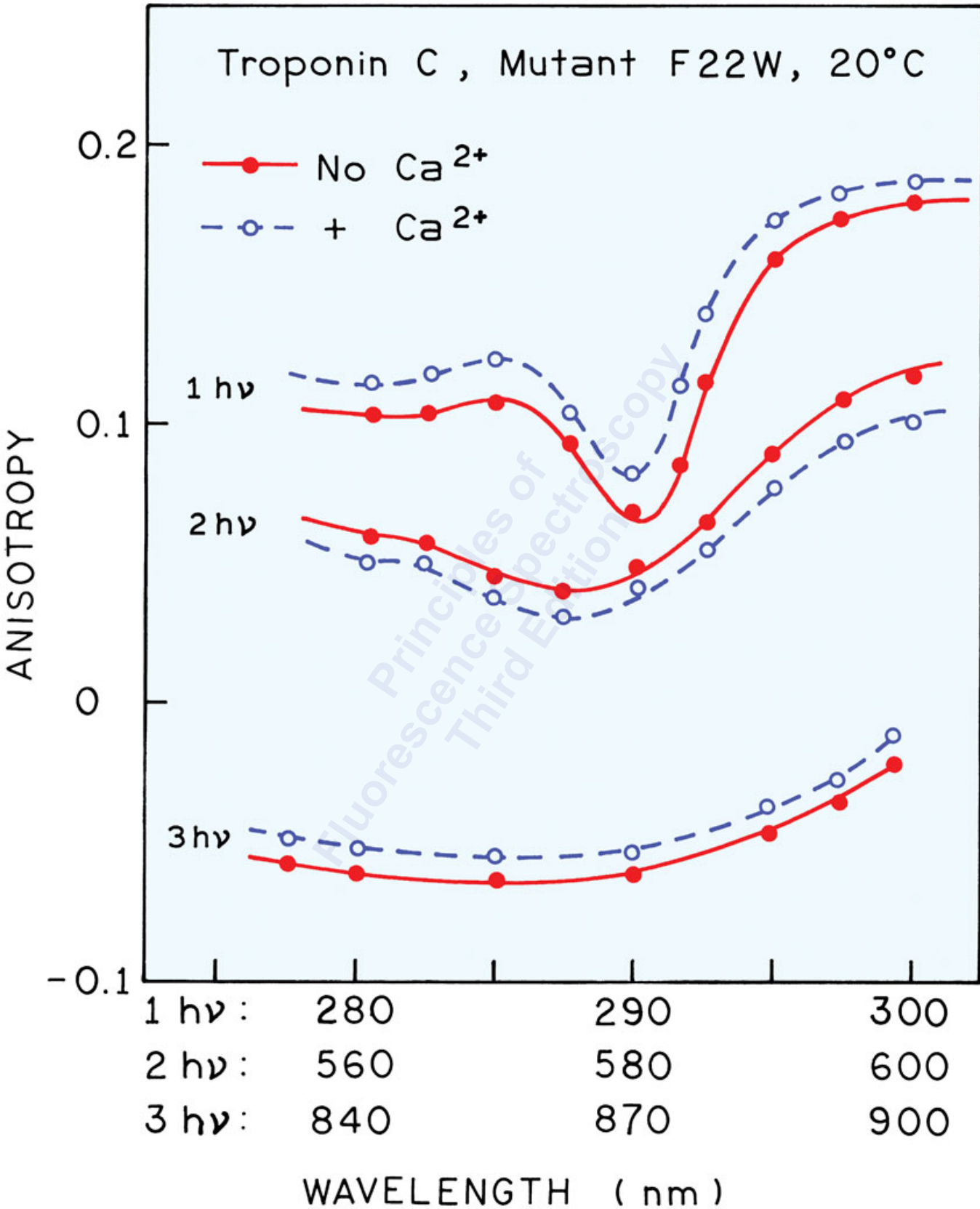
TIME (ns)



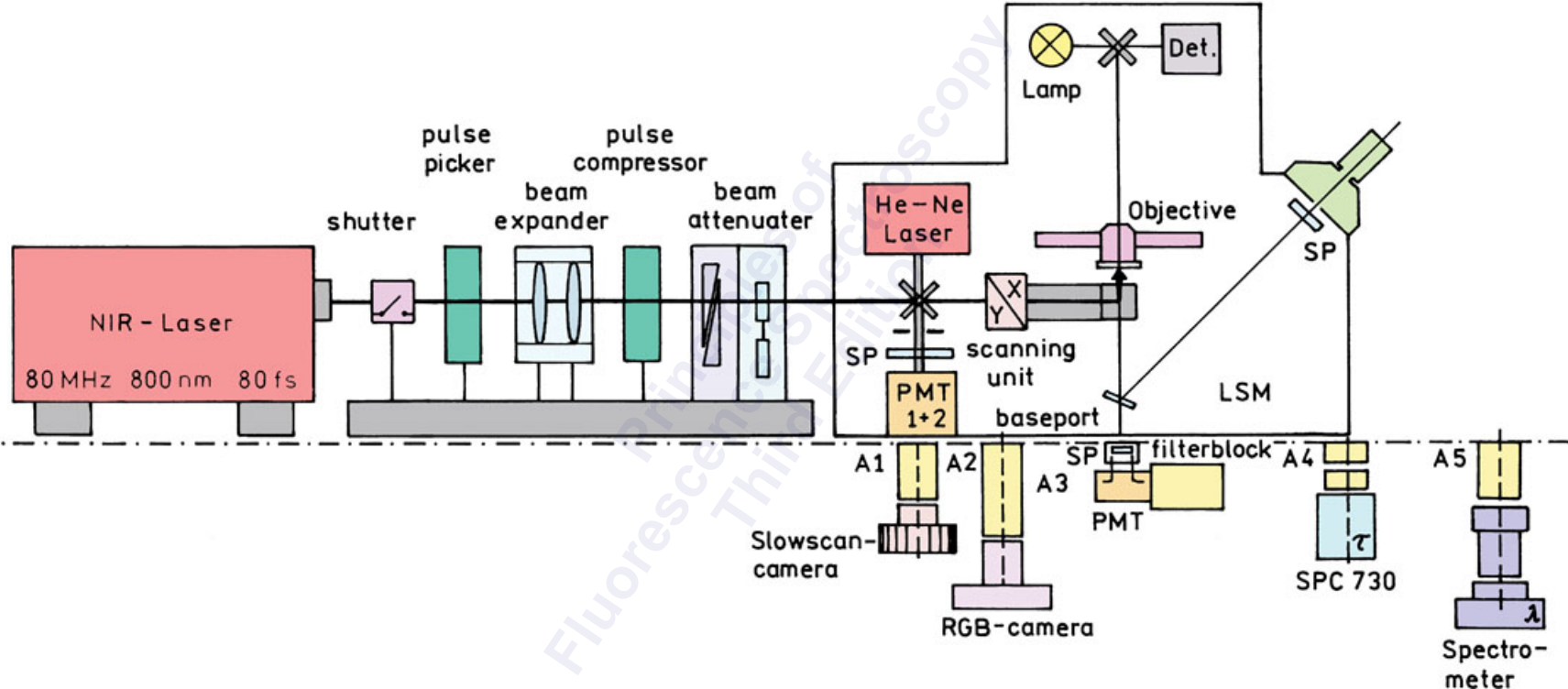








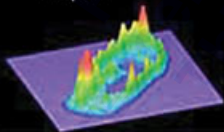




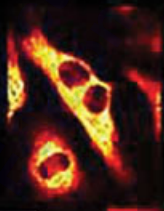
Calcium Green-C18



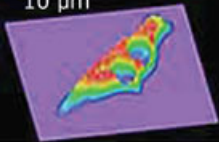
20  $\mu\text{m}$

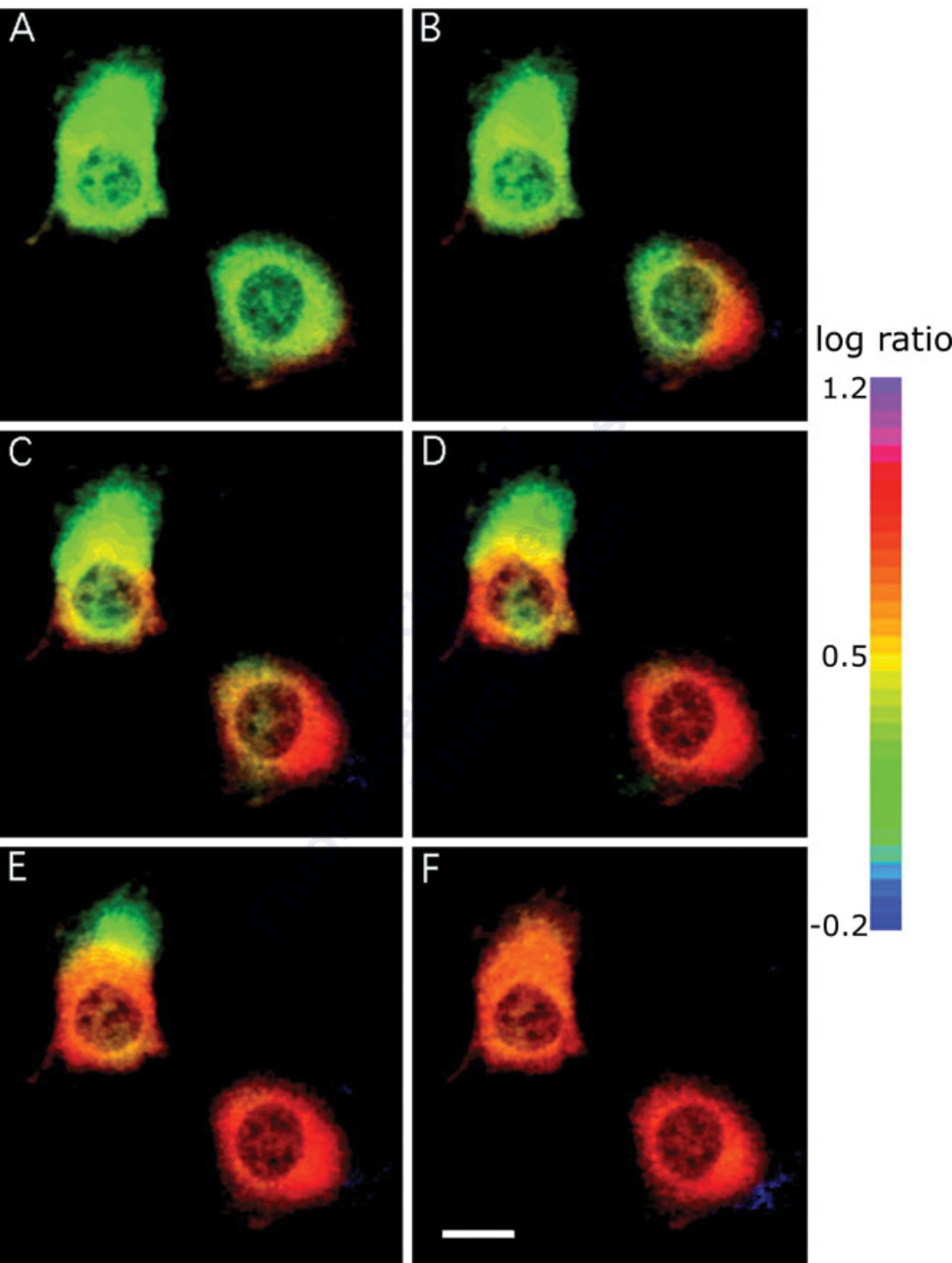


Indo-1-C18

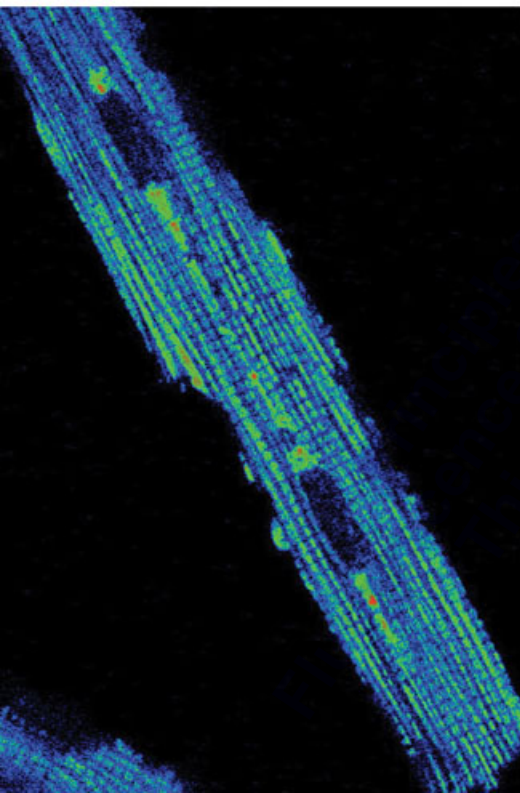


10  $\mu\text{m}$

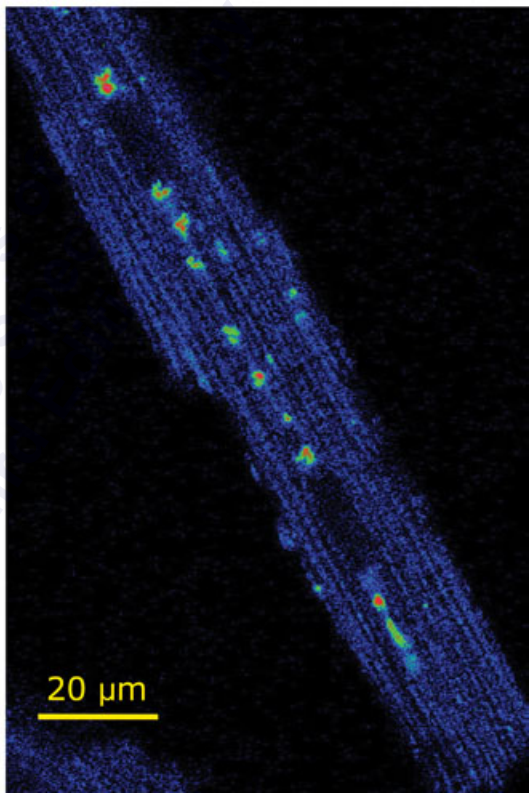




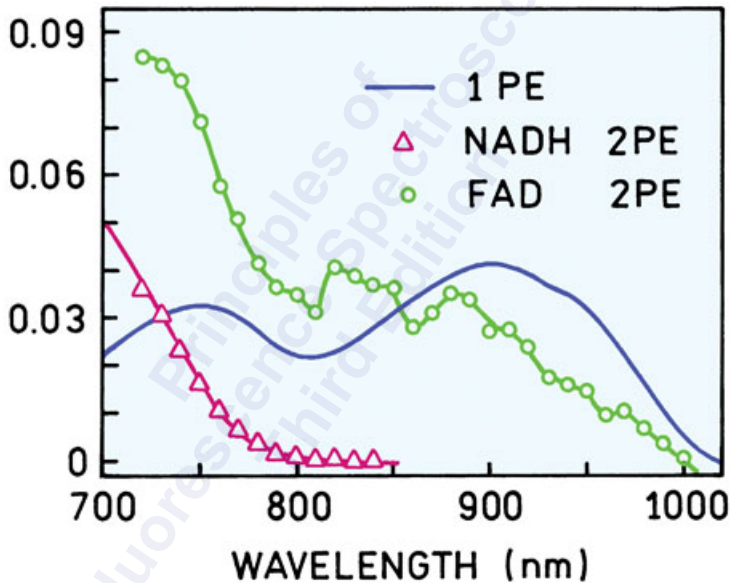
410 - 490 nm

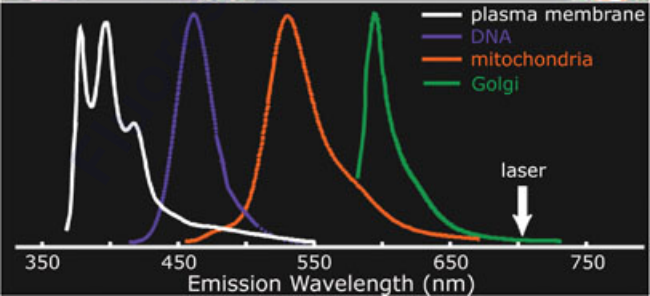
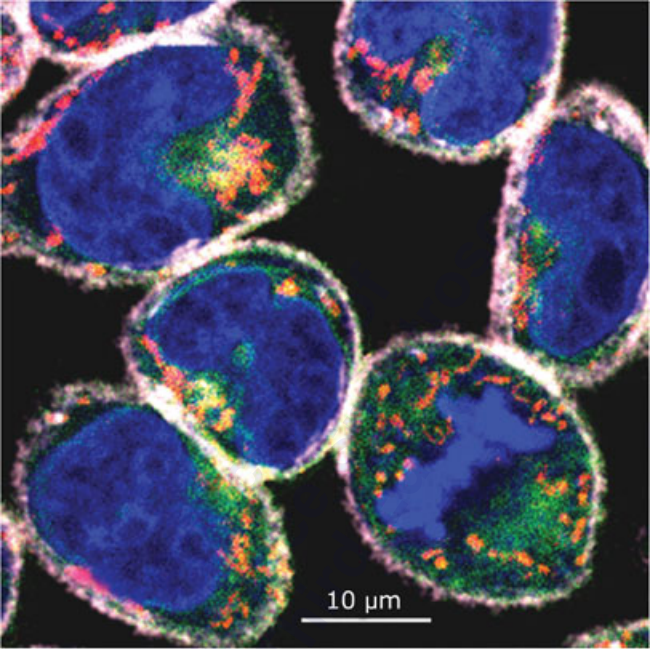


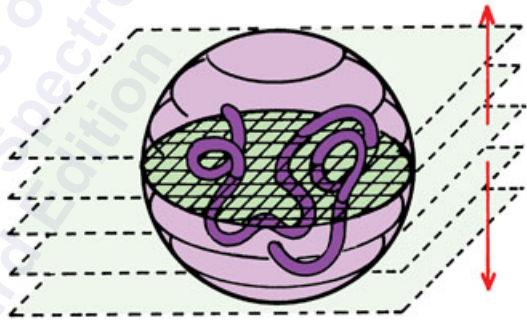
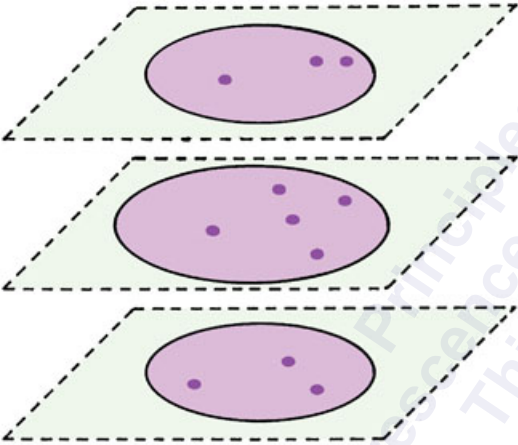
510 - 650 nm



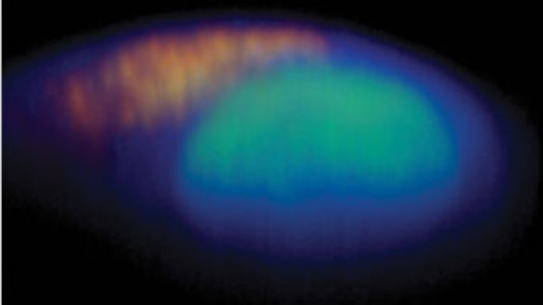
$\sigma_{2P}$  of NADH & FAD (GM)



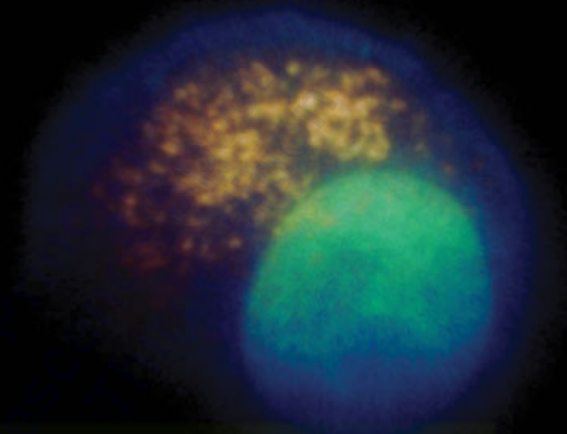




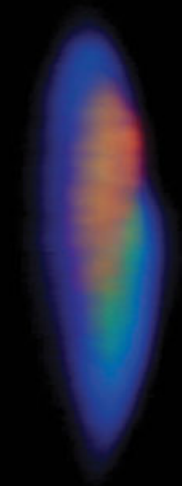
10 $\mu$ m



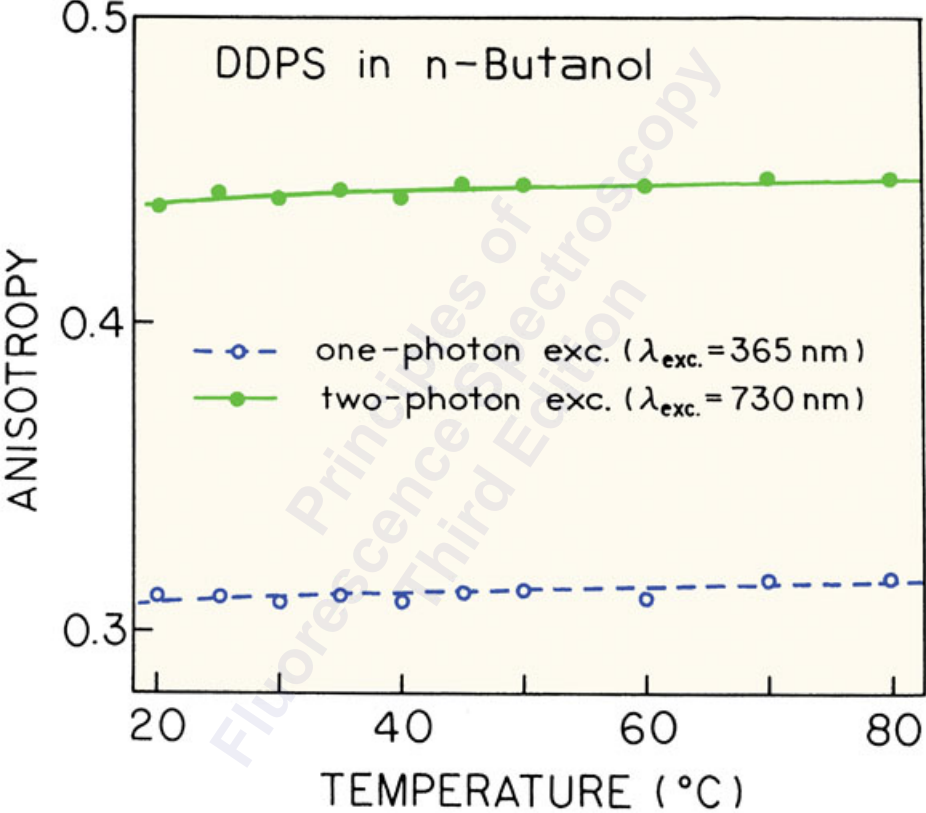
Acridine orange-DNA



Acridine orange-Acidic organelle







pH

7.52

pO<sub>2</sub>

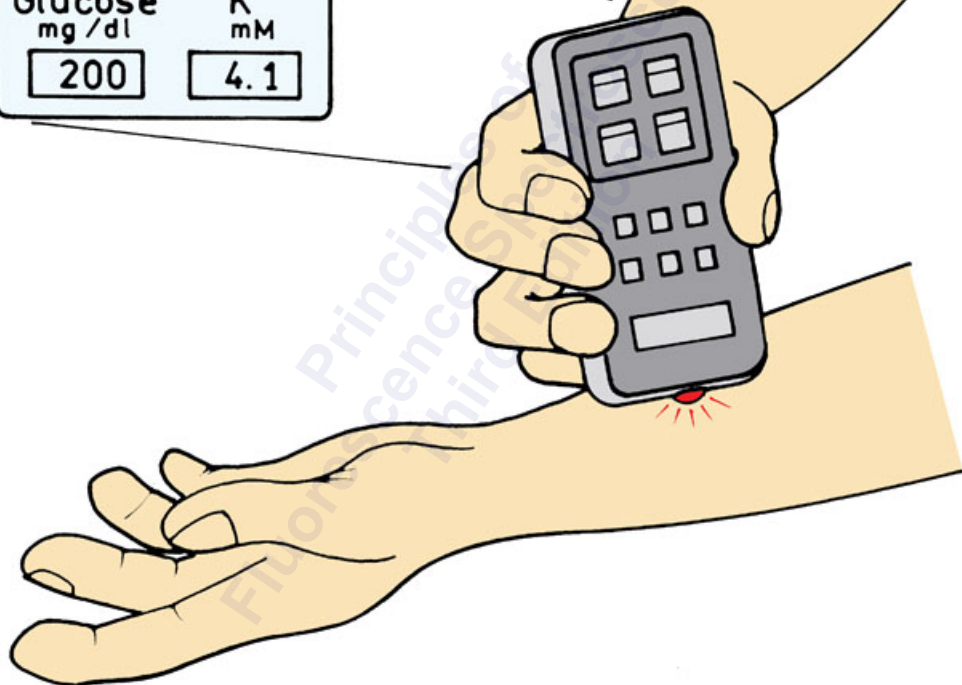
95%

Glucose  
mg/dl

200

K<sup>+</sup>  
mM

4.1

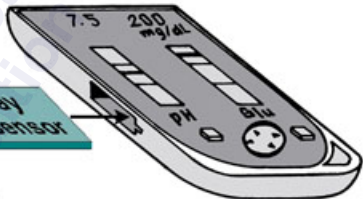


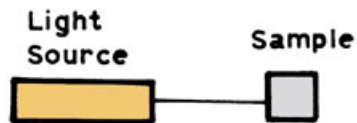
Light  
Source

$pCO_2$   $pO_2$  pH  
 $Ca^{2+}$   $Mg^{2+}$   $K^+$   
Cardiac Markers  
Immunoassays

Array  
Sensor

Array  
Detector





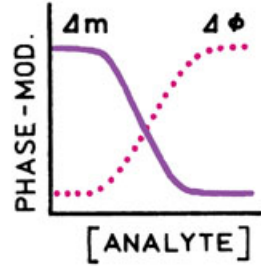
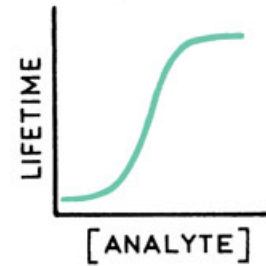
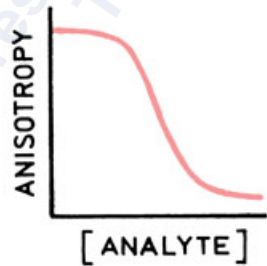
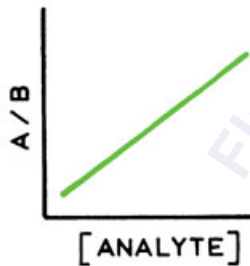
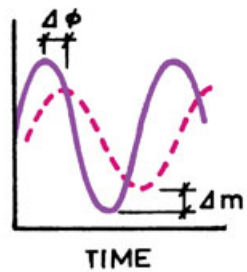
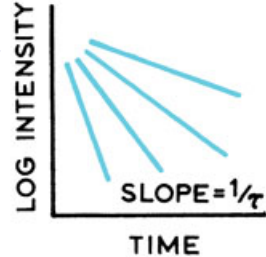
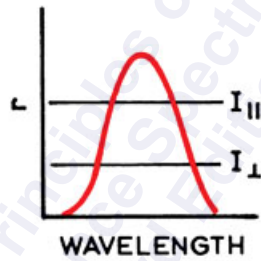
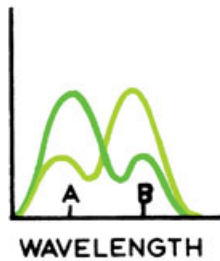
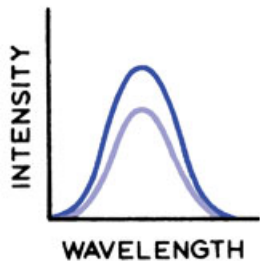
Intensity

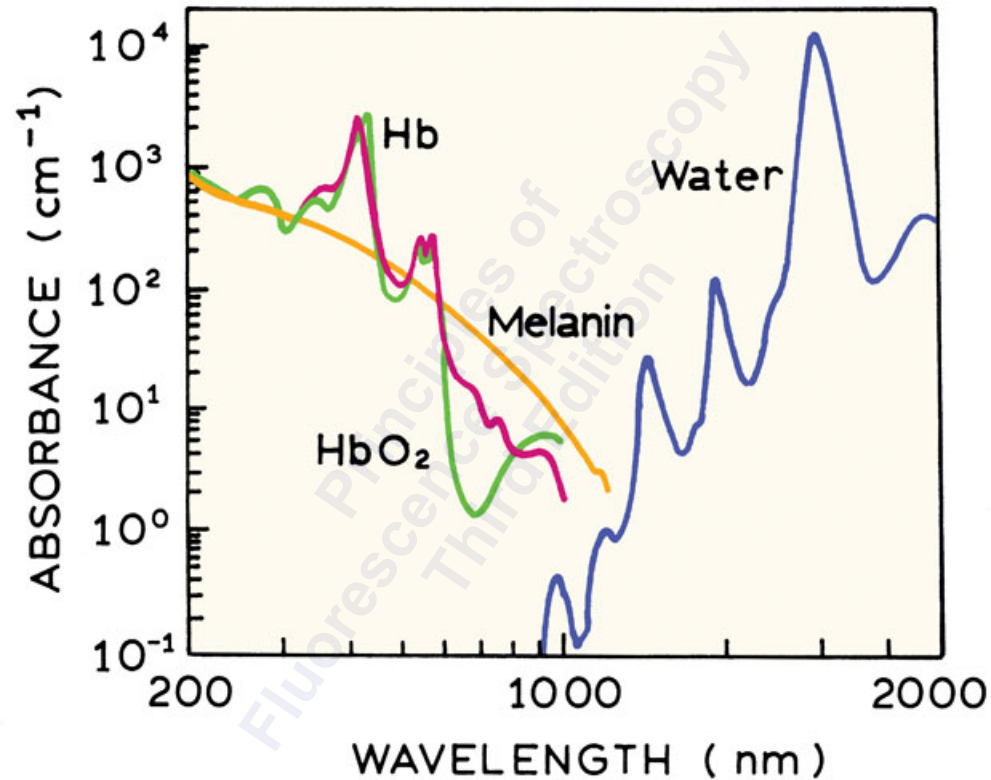
Intensity Ratio

Anisotropy

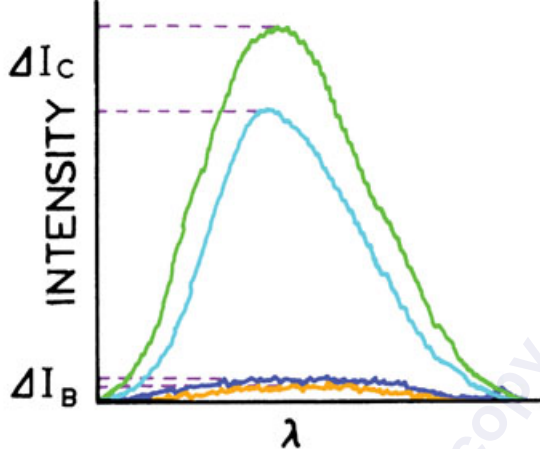
Time

Phase - Modulation





# Steady State Fluorescence



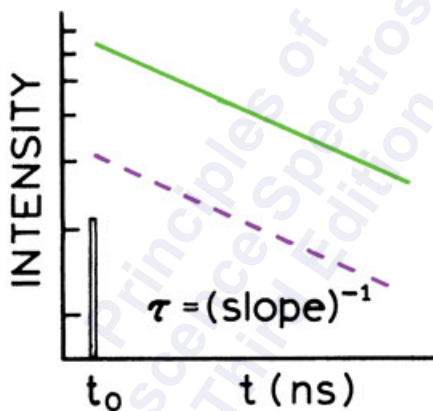
Cuvette



Syringe

# Time-Resolved Fluorescence

## Time-Domain

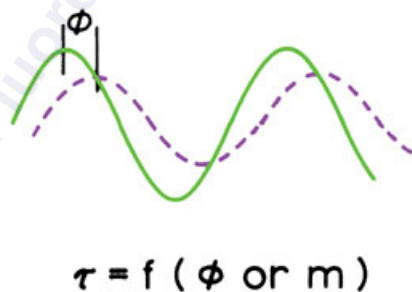


Cuvette



Syringe

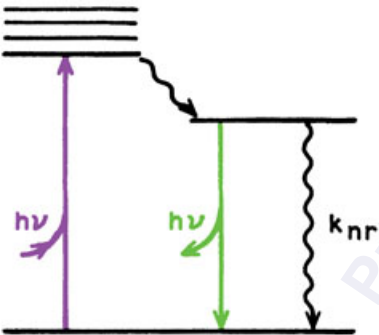
## Frequency-Domain



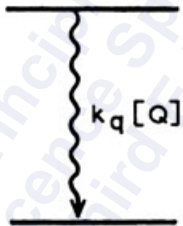
Cuvette



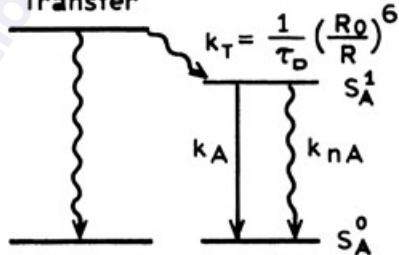
Syringe



**Collisional Quenching**

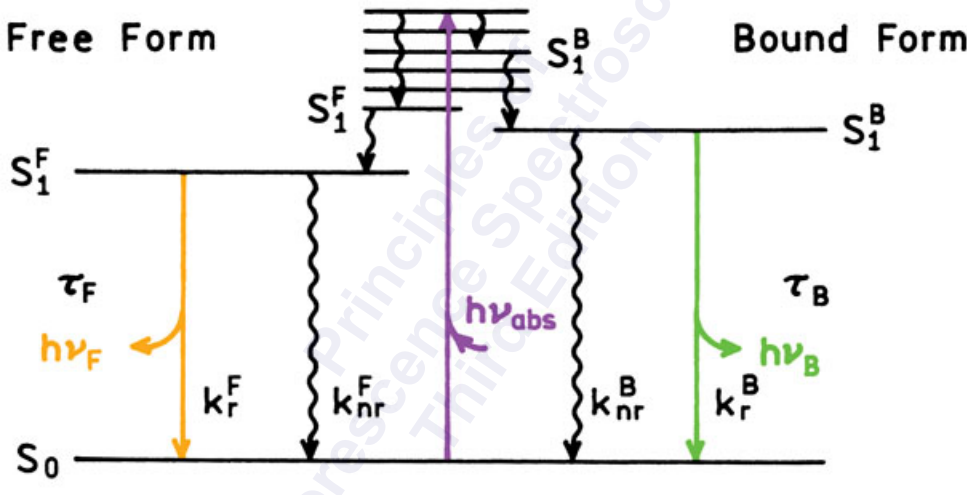


**Energy Transfer**

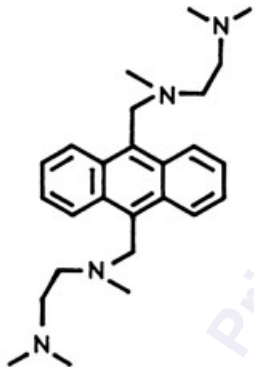


Free Form

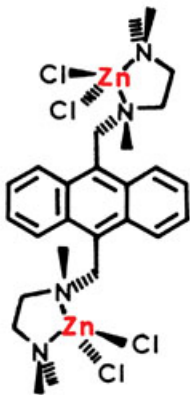
Bound Form



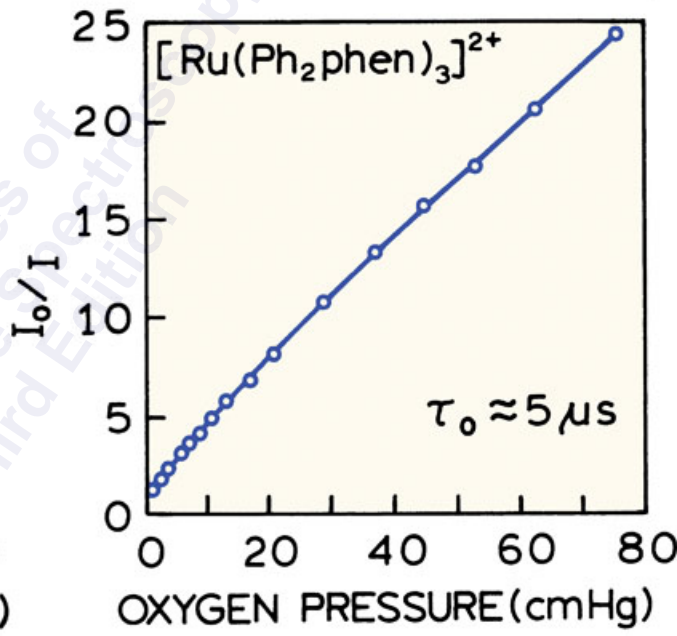
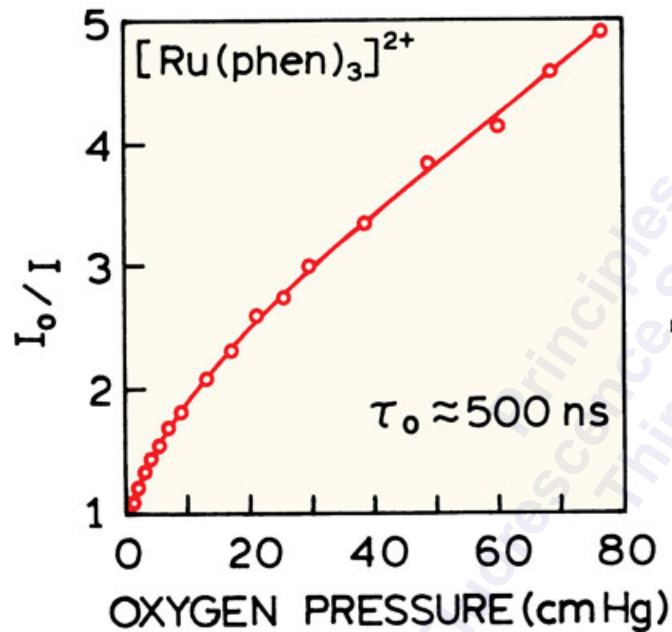


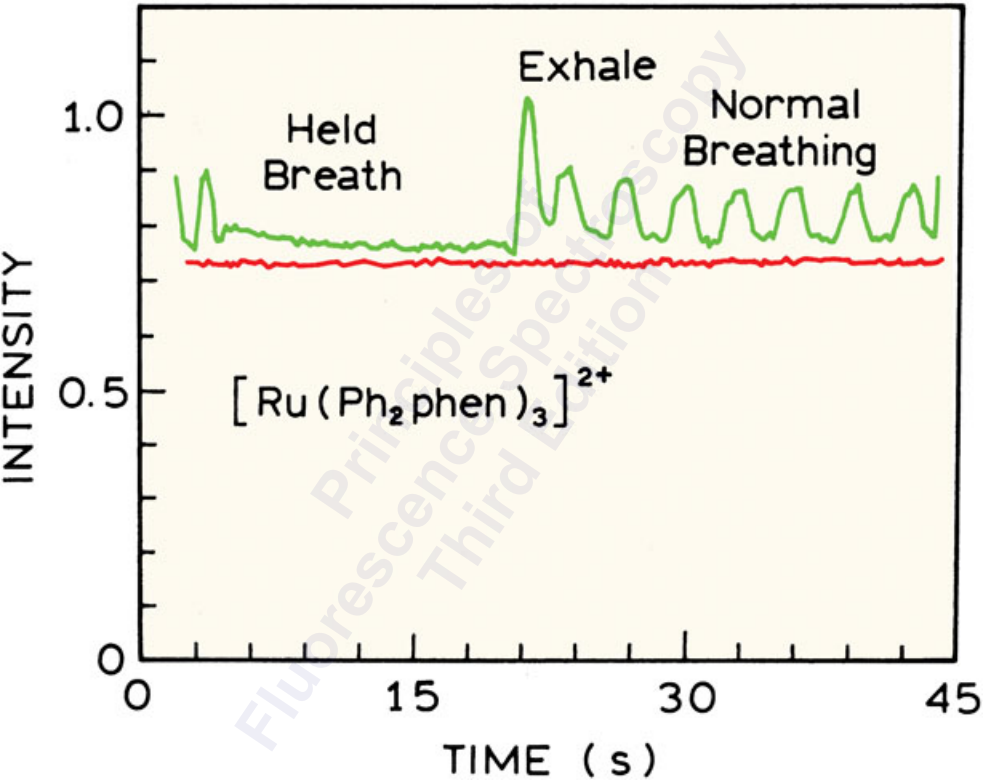


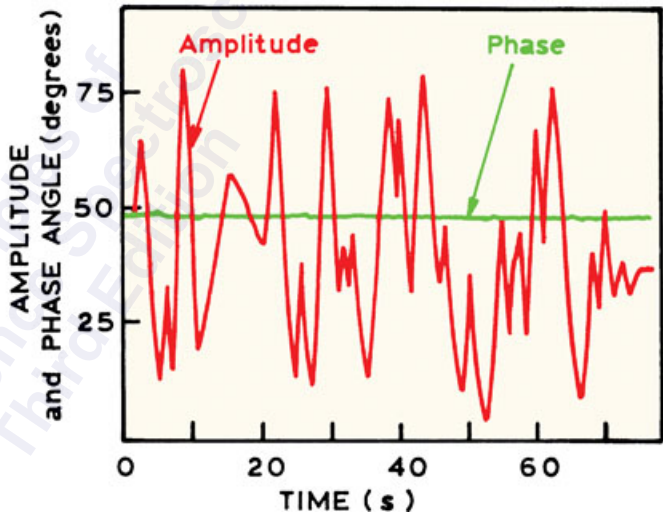
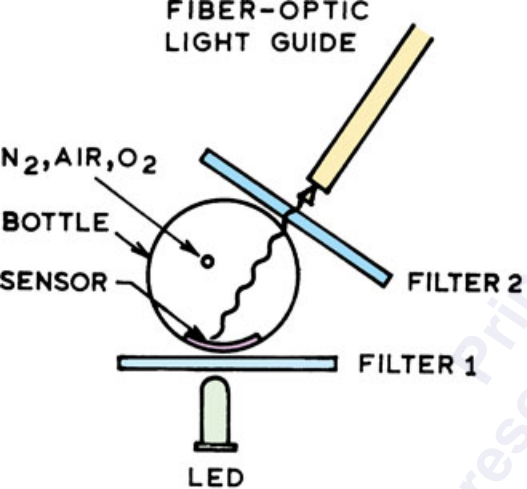
Non-fluorescent

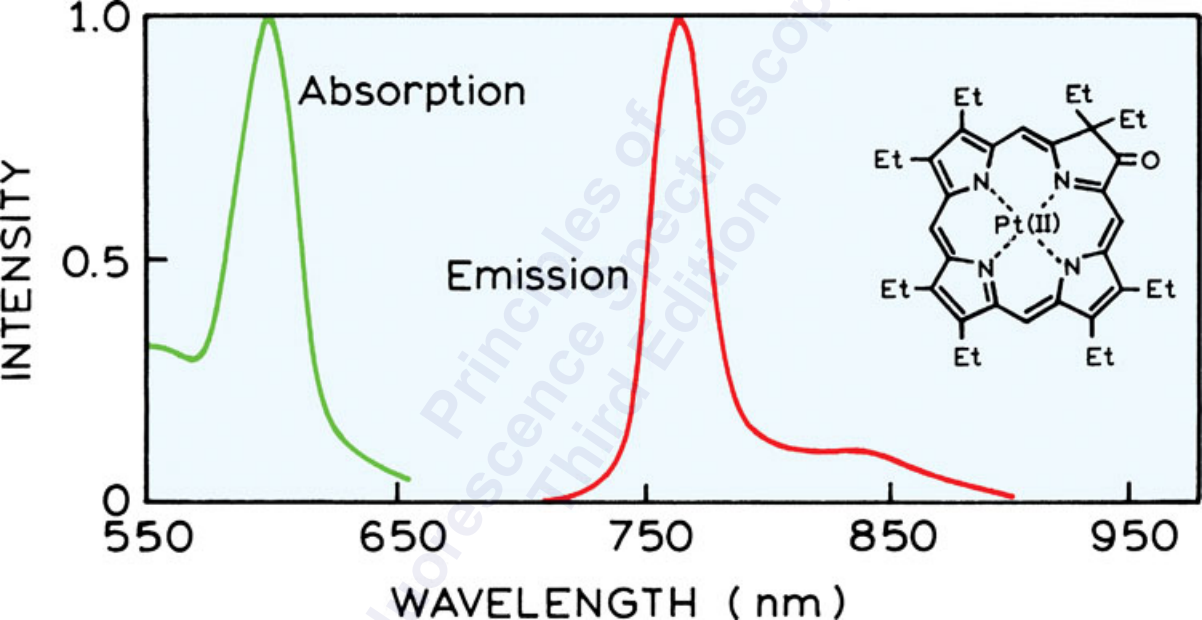


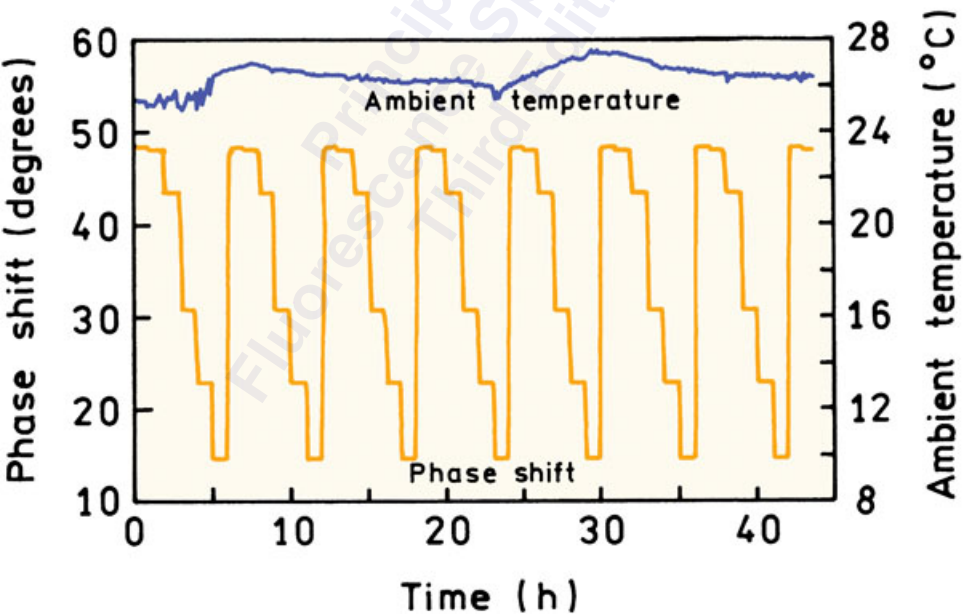
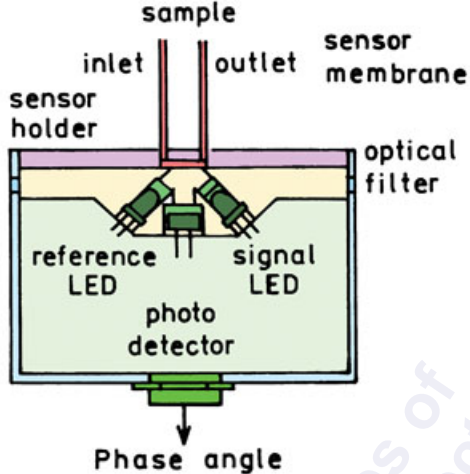
Fluorescent

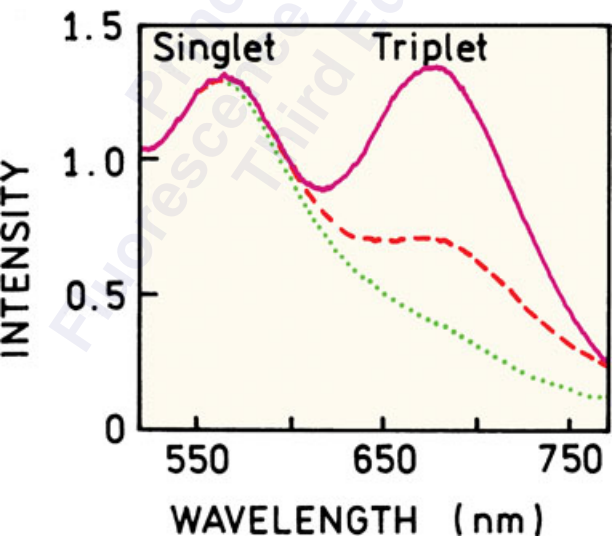
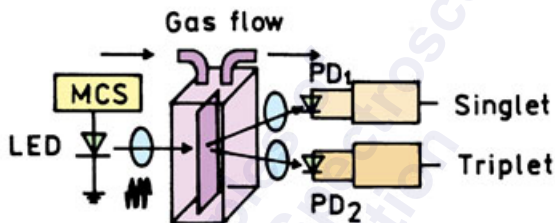
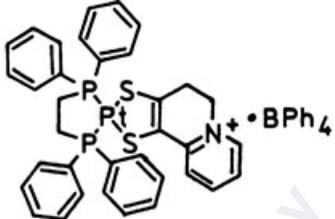


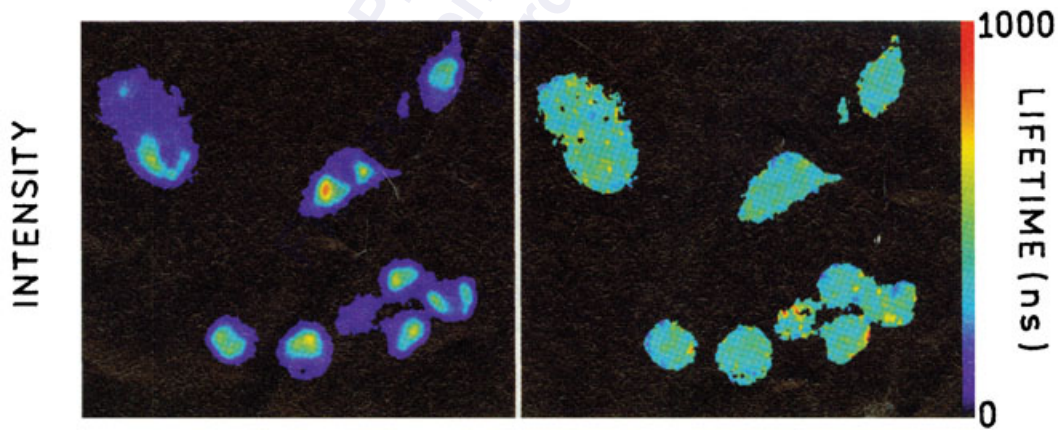
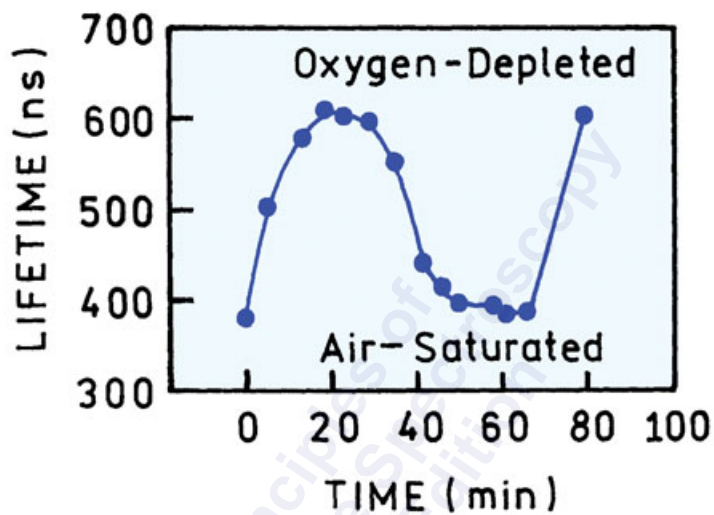




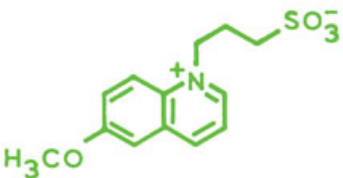




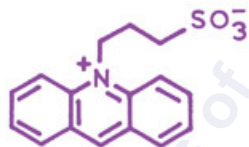




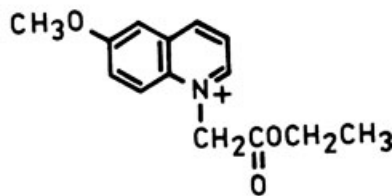




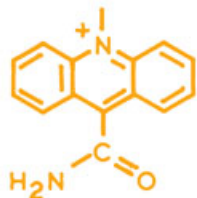
SPQ



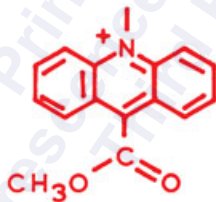
SPA



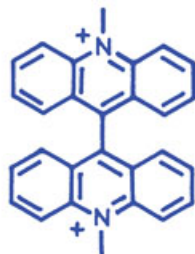
MQAE



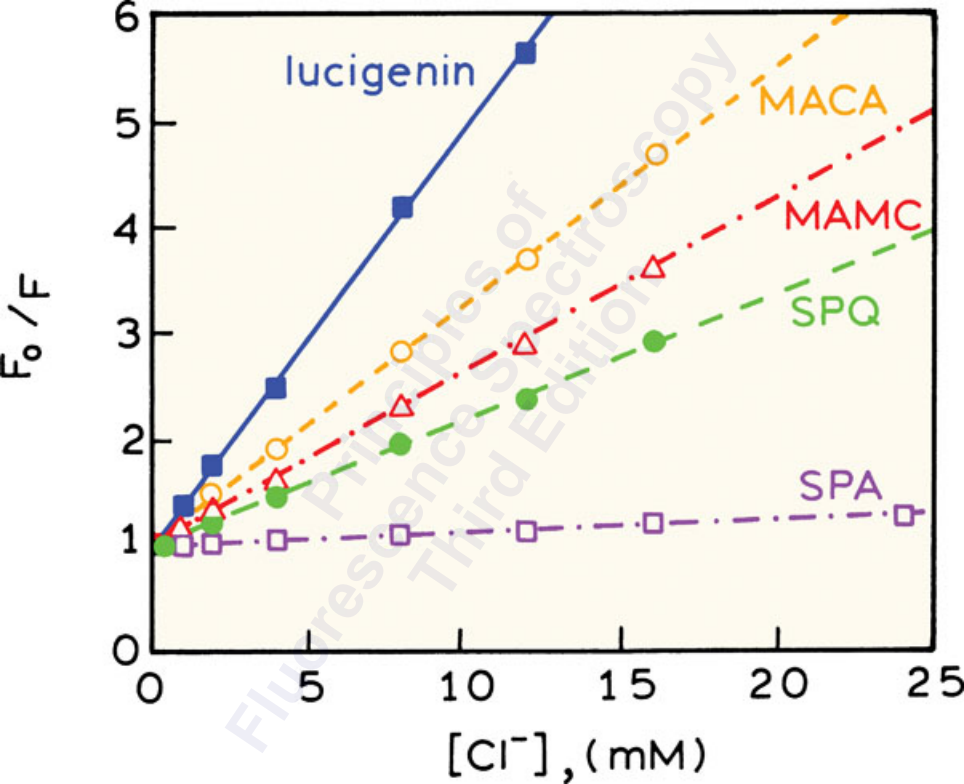
MACA



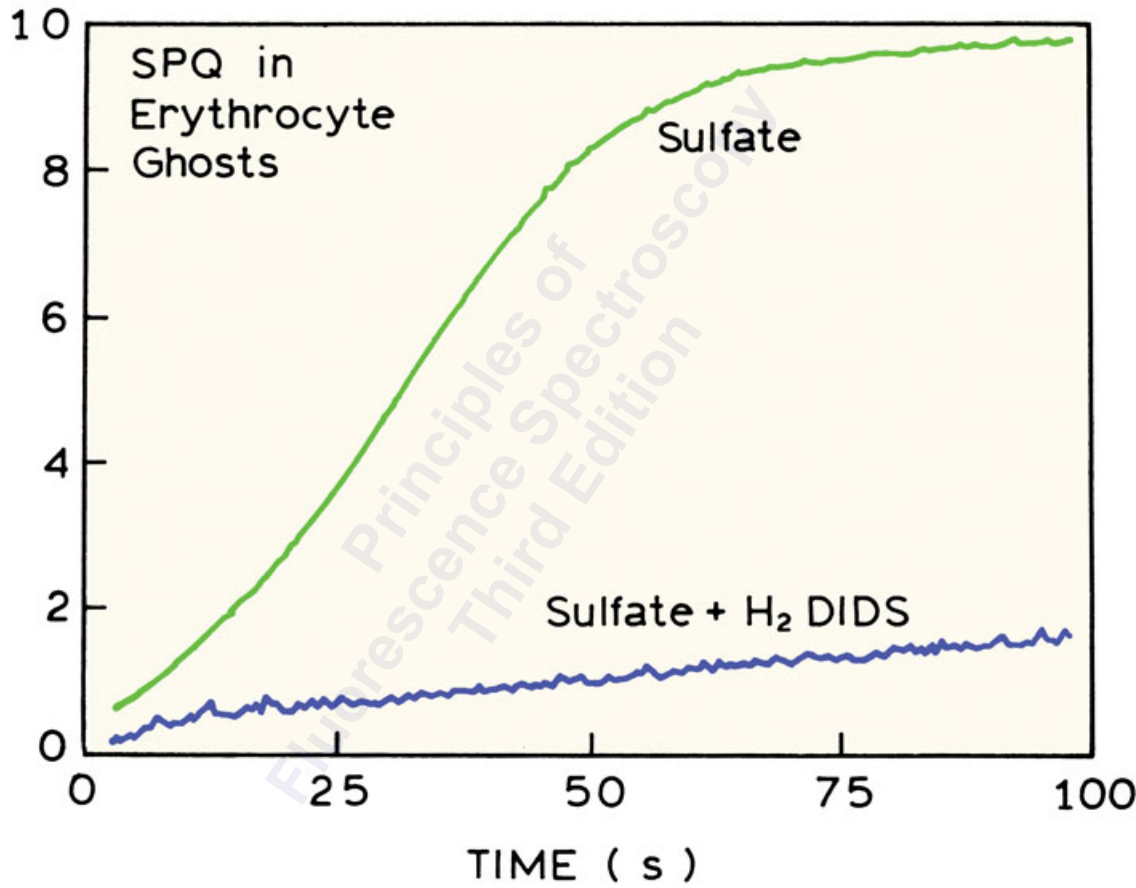
MAMC

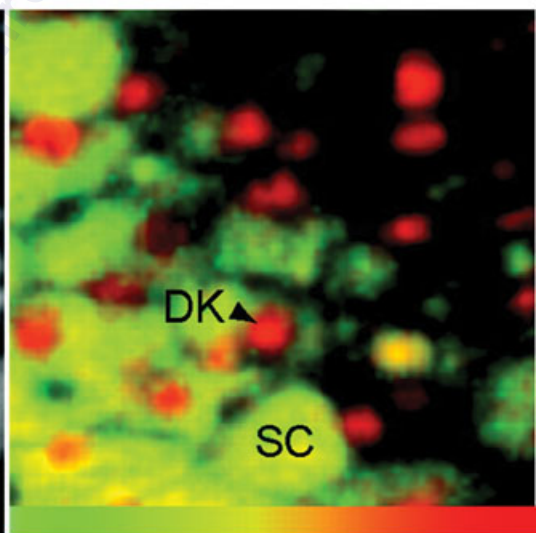
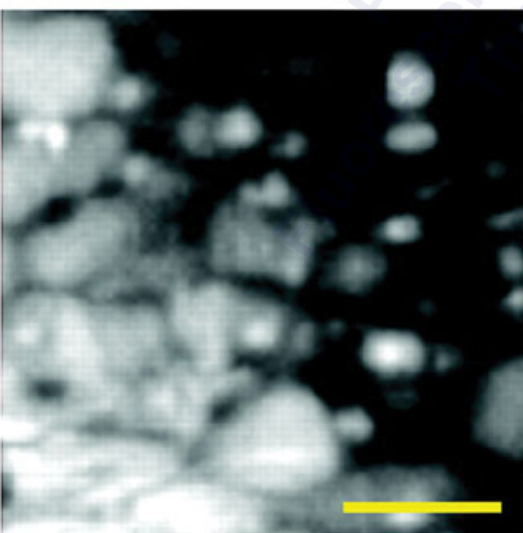
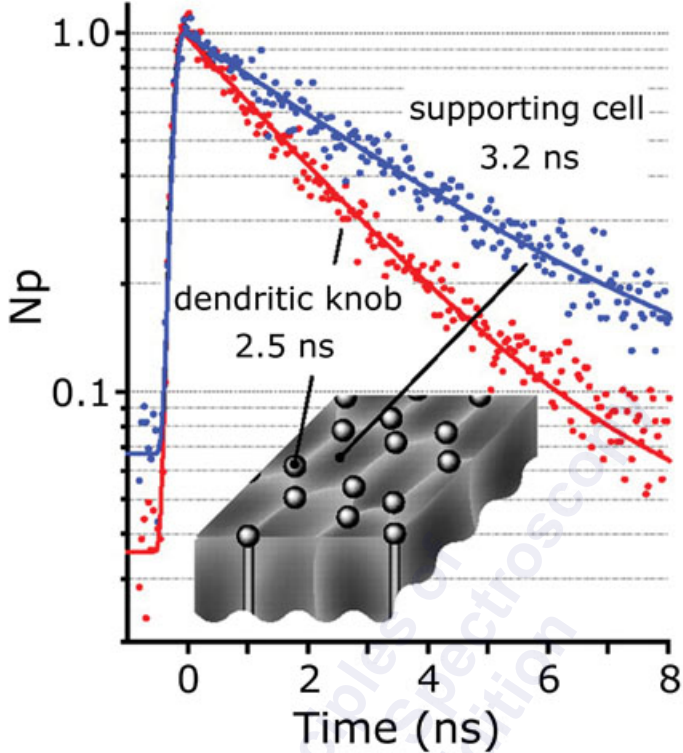


Lucigenin

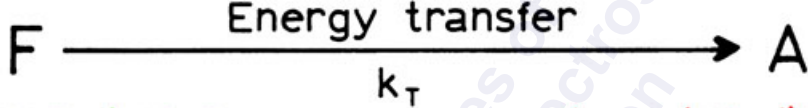


FLUORESCENCE (Arbitrary Units)



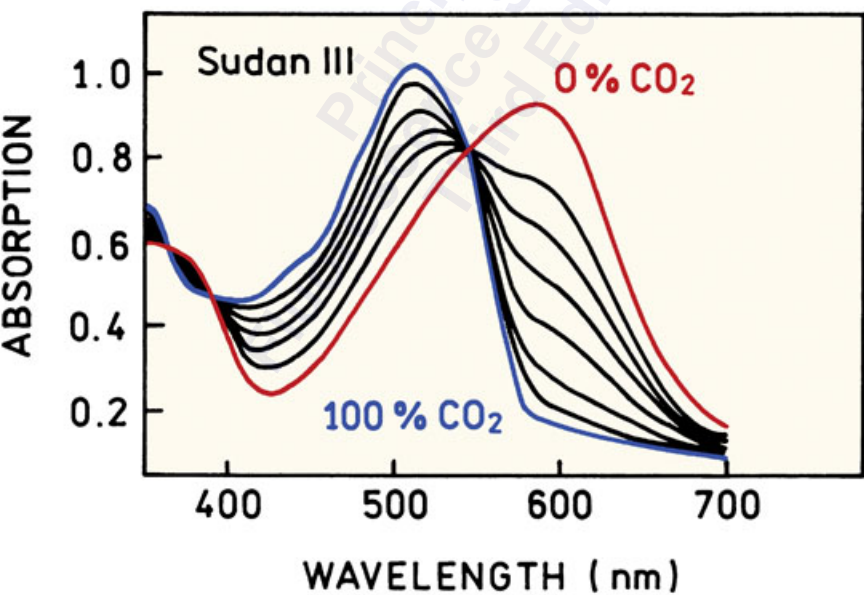
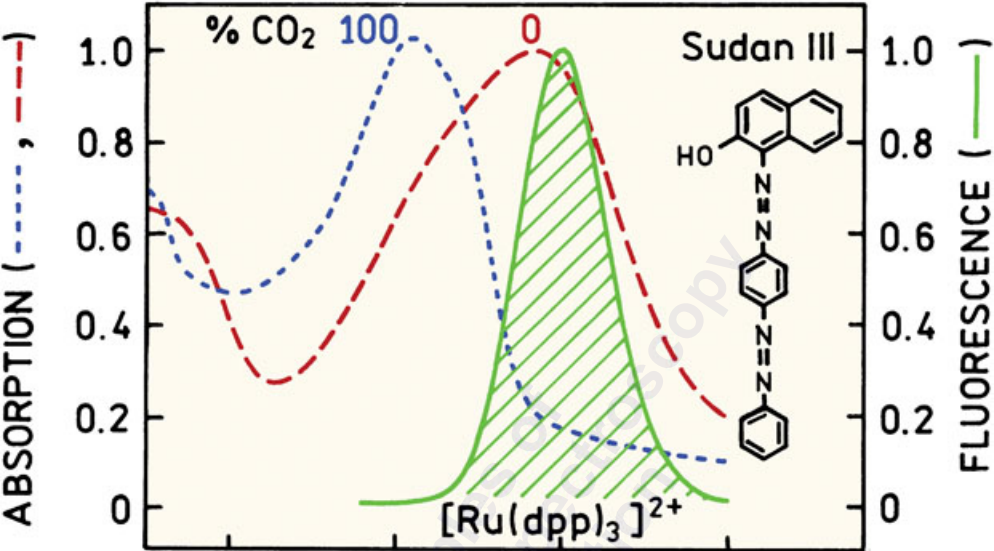


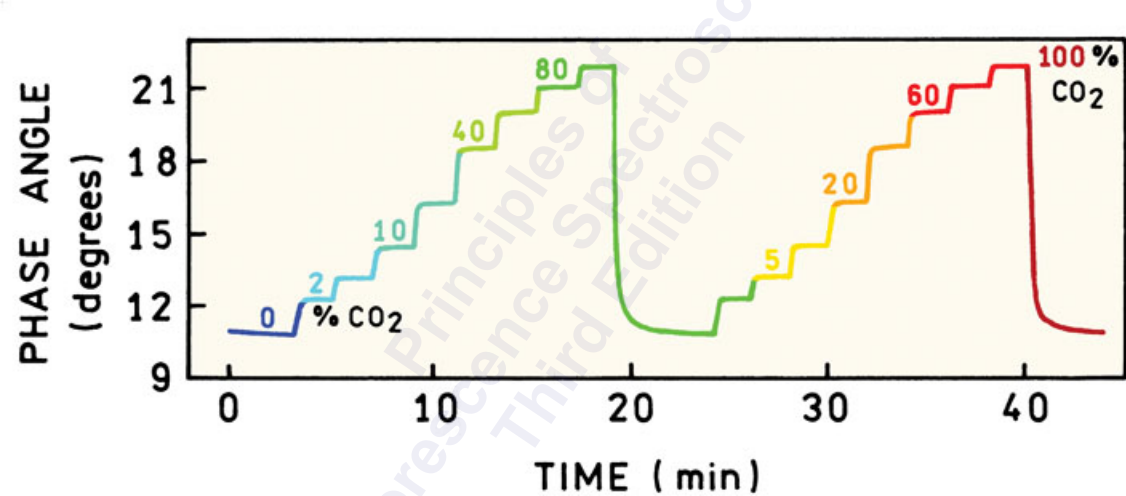
20 30 40 50 60 70  
 $[Cl^-]_i$  (mM)

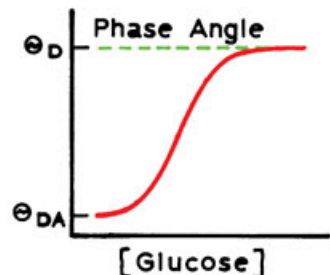
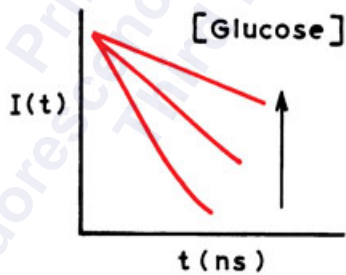
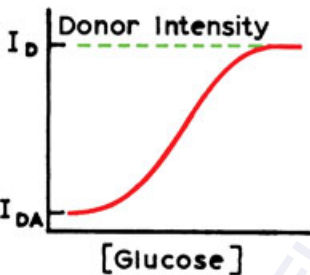


Donor chosen  
for use with  
simple  
light sources,  
desired spectra  
and lifetime.

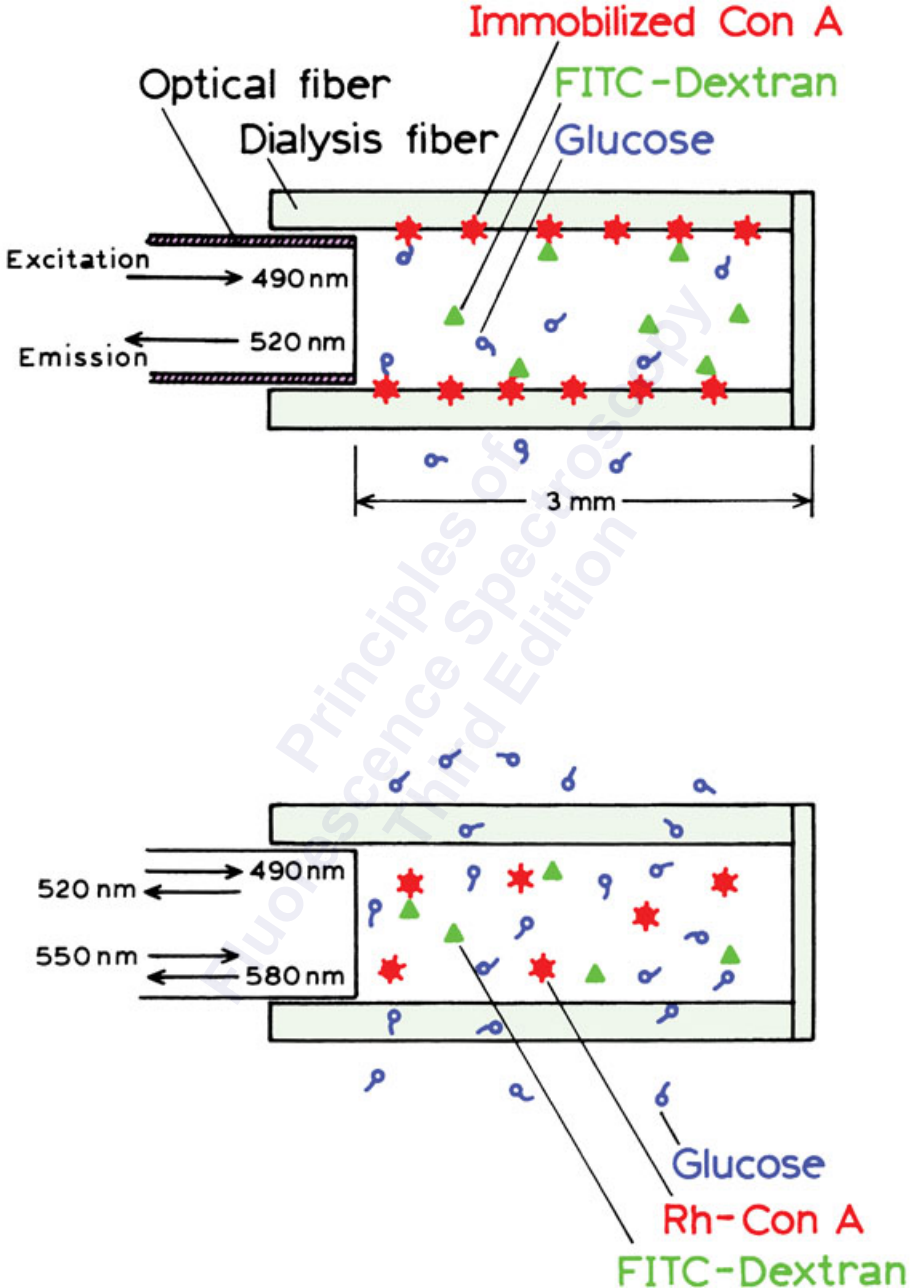
Acceptor displays  
changes in its  
absorption spectrum  
in response to analyte.





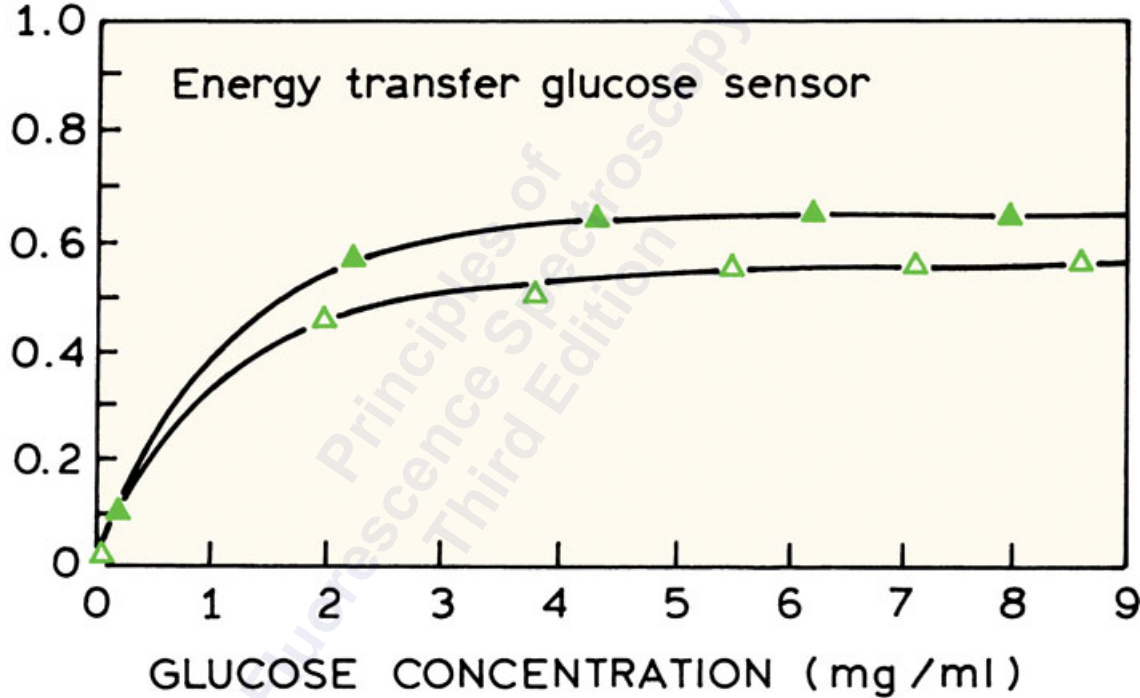


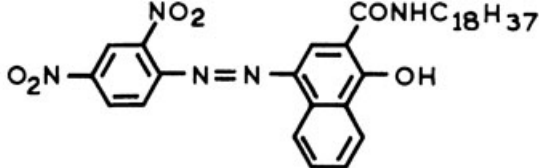




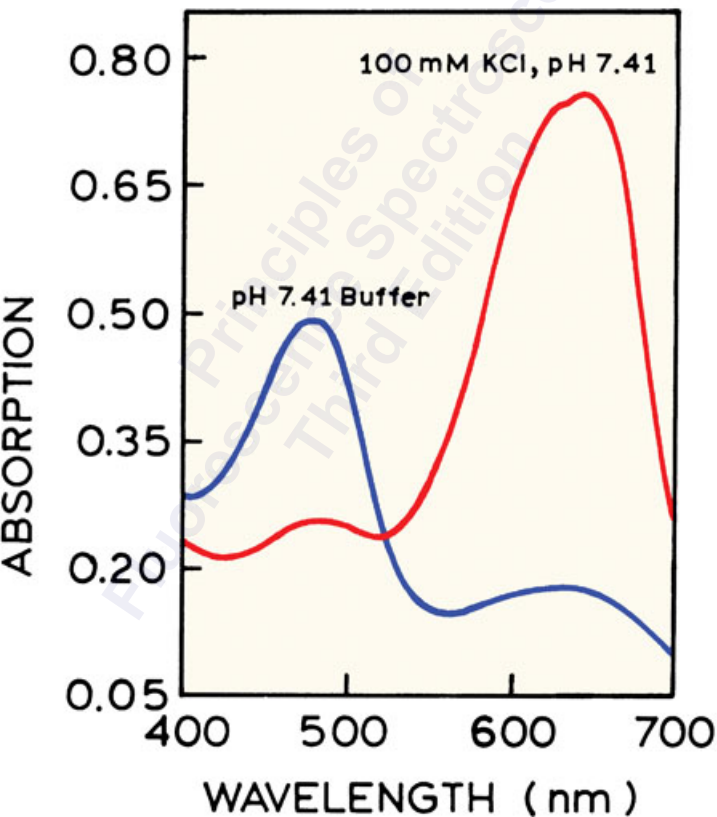
FRACTIONAL RECOVERY OF

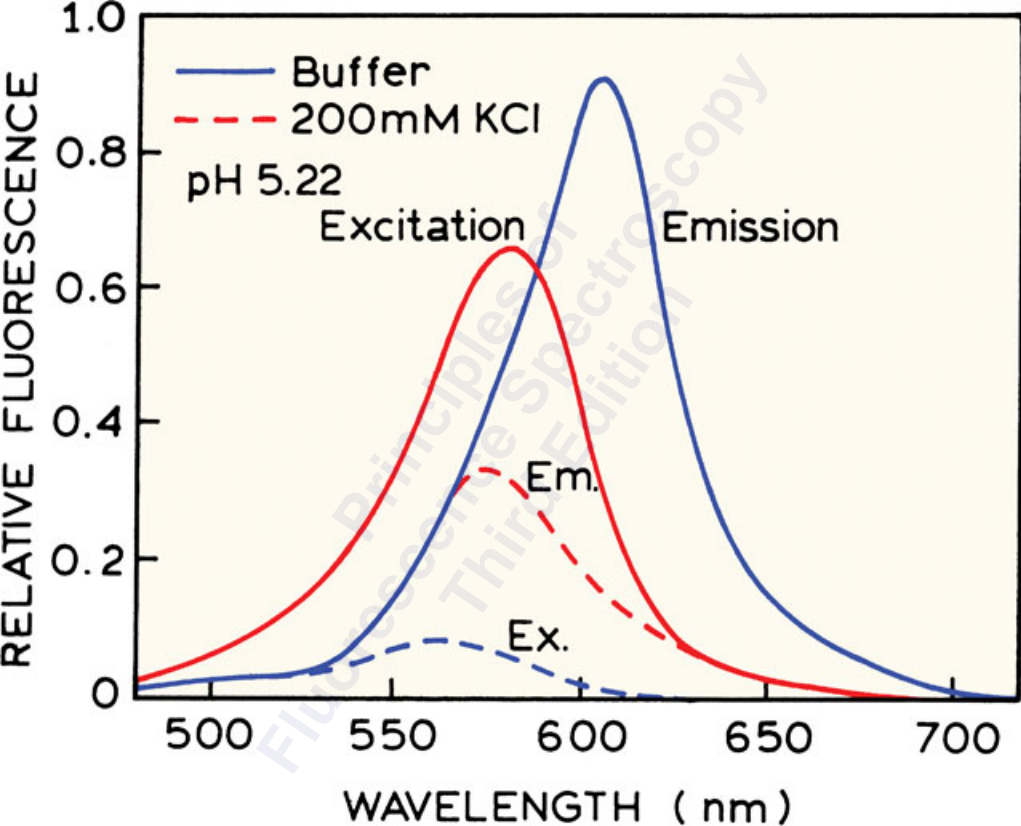
FITC FLUORESCENCE

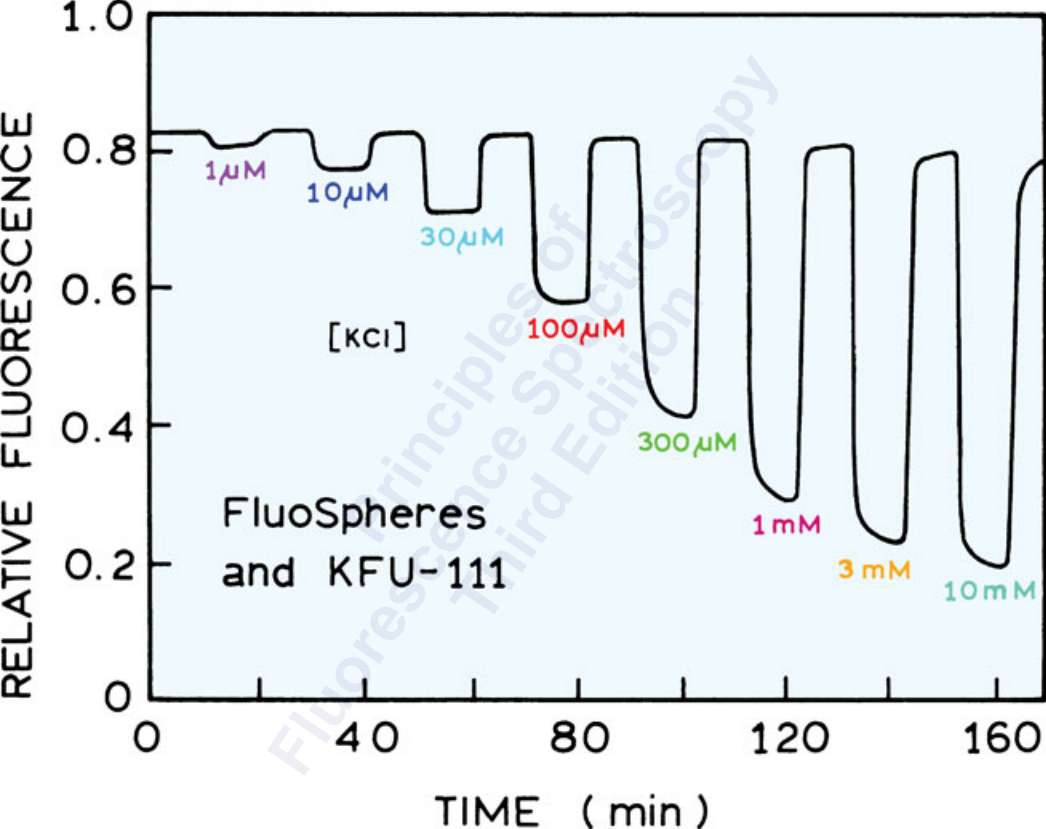


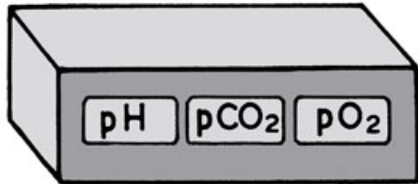


KFU 111

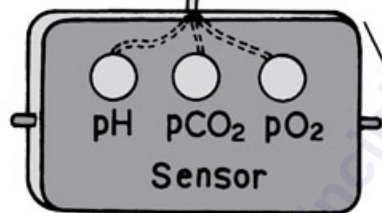




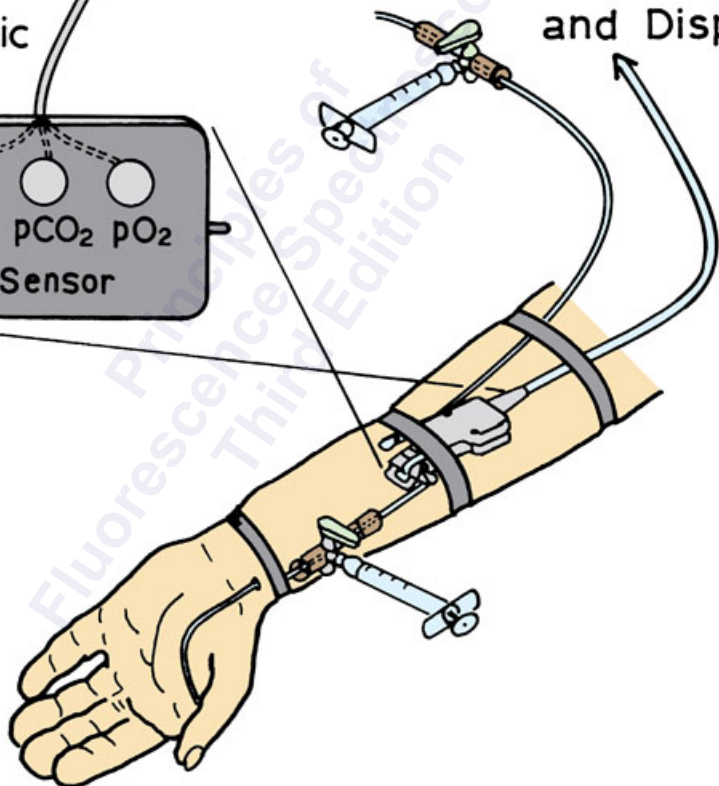


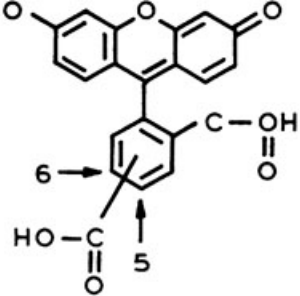


Display  
Fiber Optic



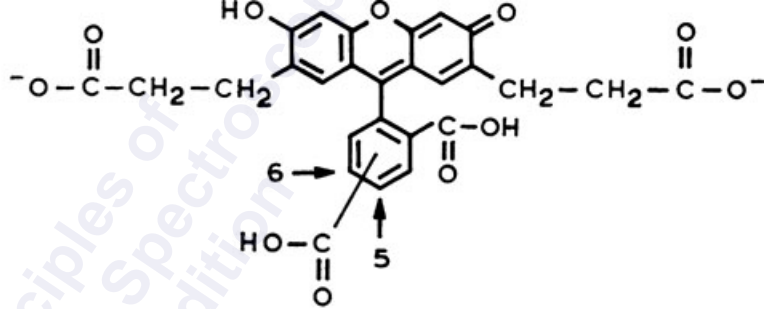
To Blood  
Gas Monitor  
and Display





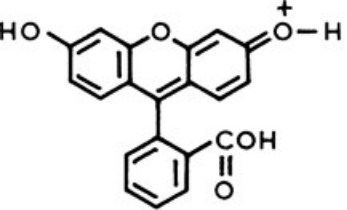
$pK_a \approx 6.5$

Fluorescein or  
5(6)-Carboxy  
fluorescein

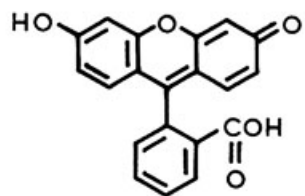
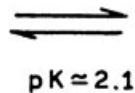


$pK_a \approx 7.0$

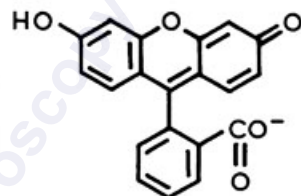
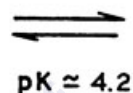
BCECF



cation

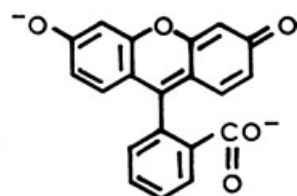
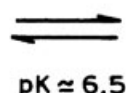


neutral



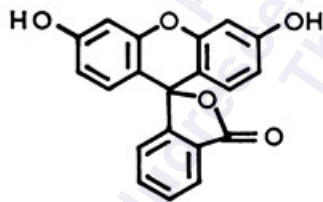
monoanion

Fluorescent



dianion

Fluorescent

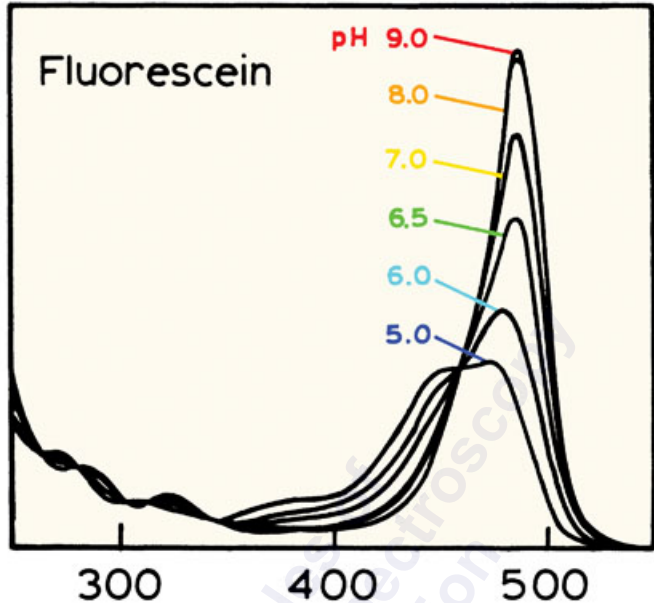


lactone



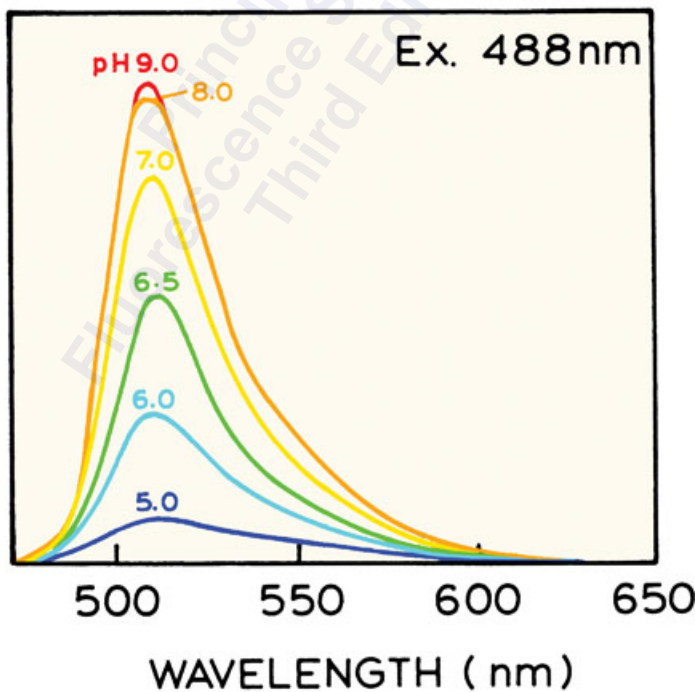
ABSORBANCE

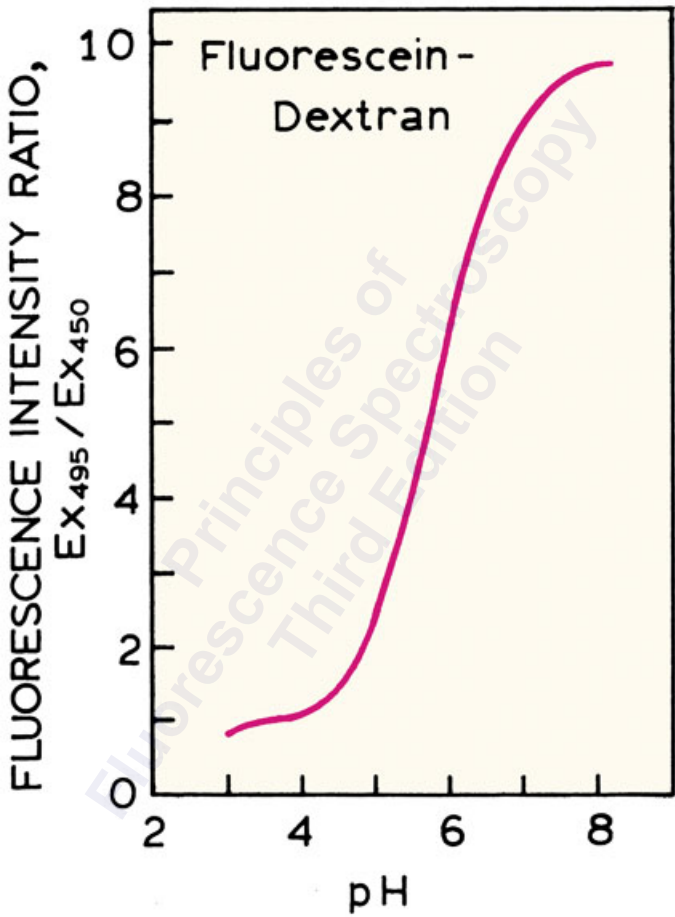
Fluorescein

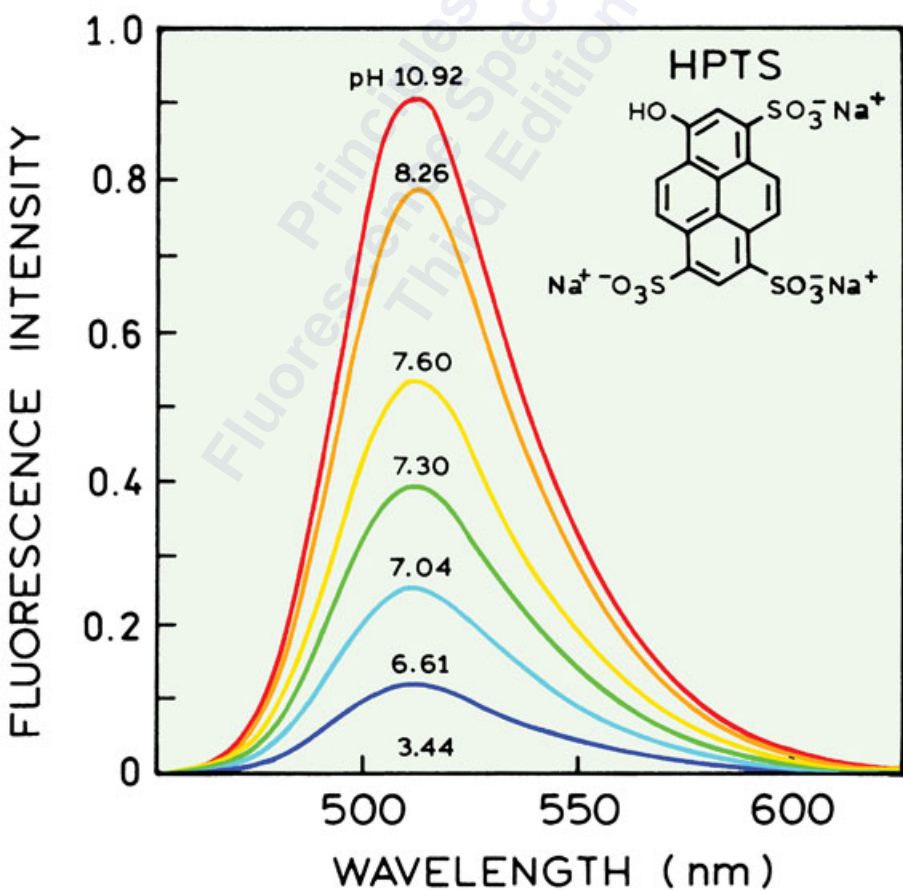
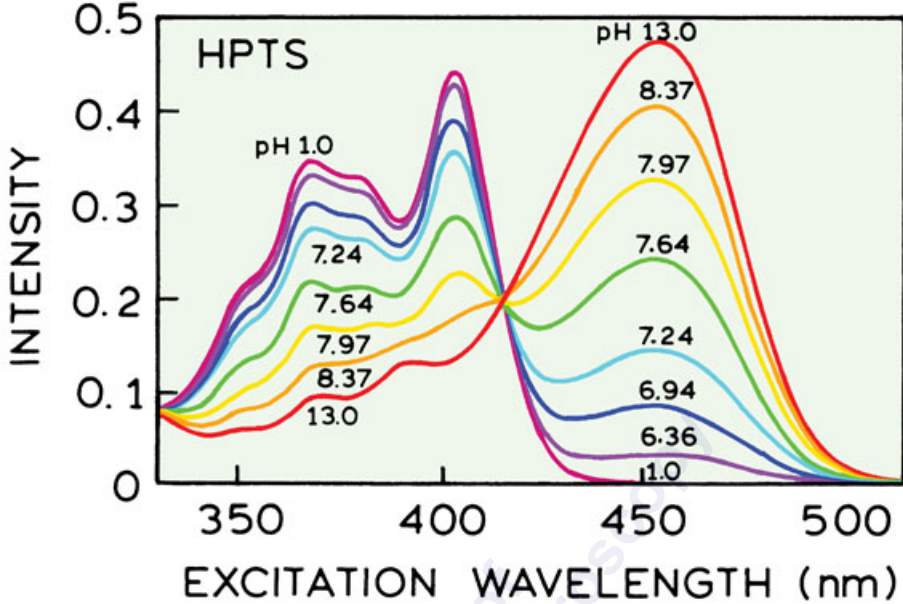


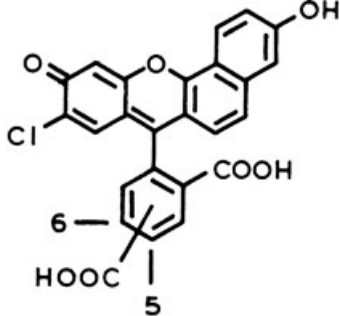
FLUORESCENCE EMISSION

Ex. 488nm

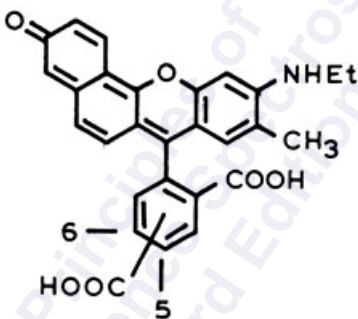




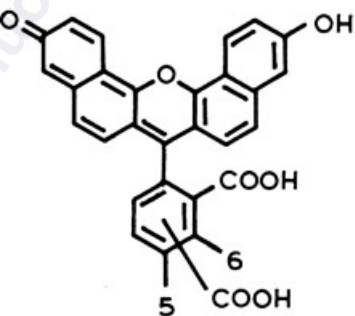




Carboxy SNAFL-2

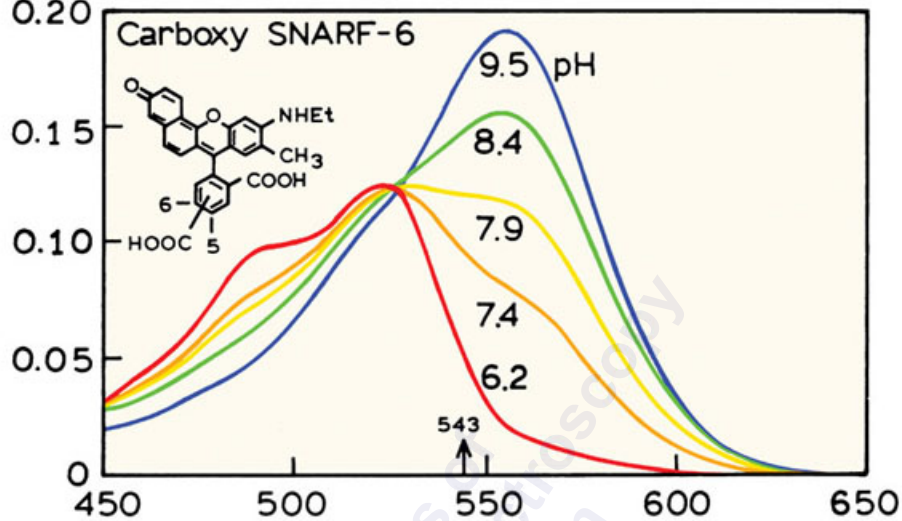


Carboxy SNARF-6

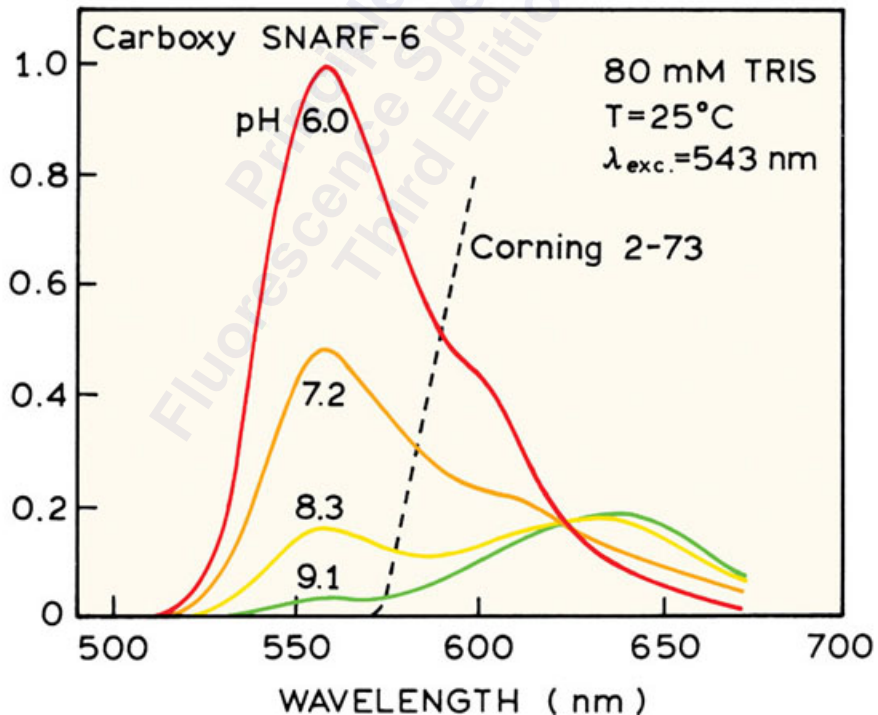


CNF

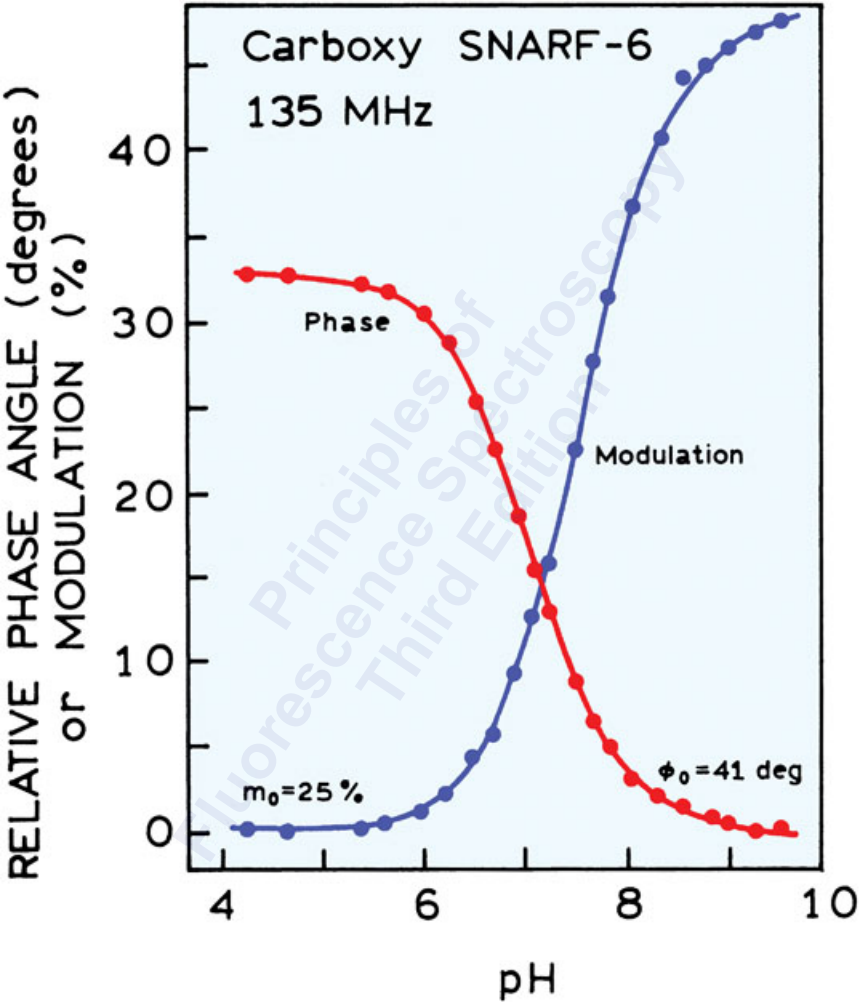
ABSORPTION

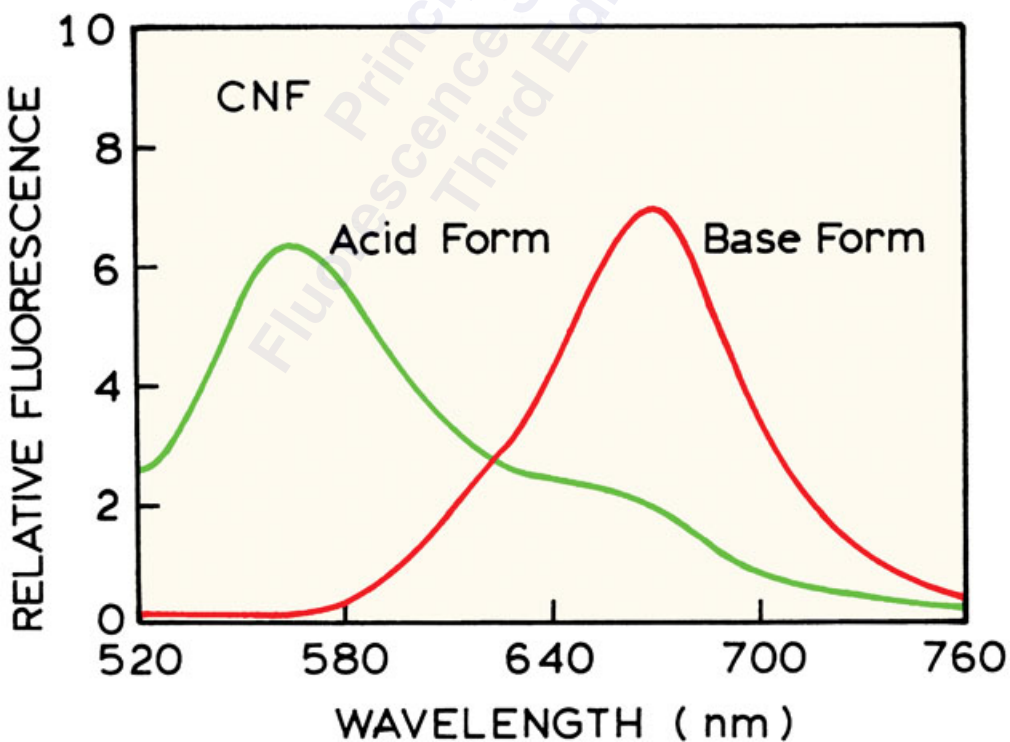
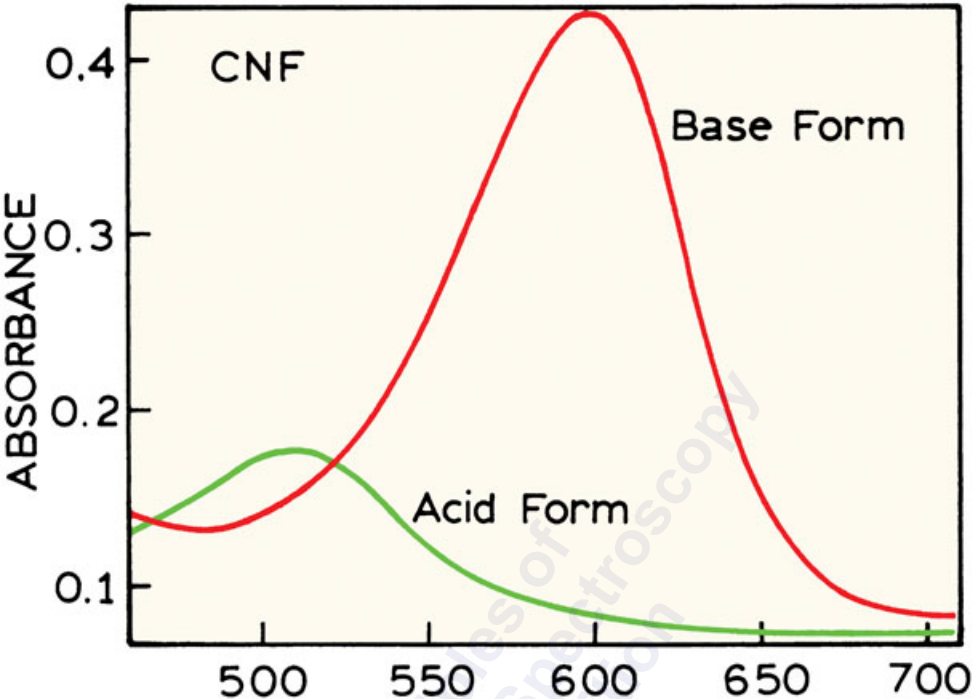


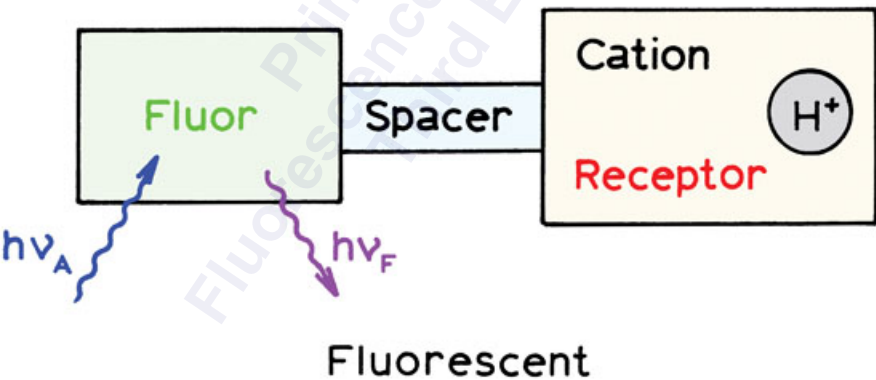
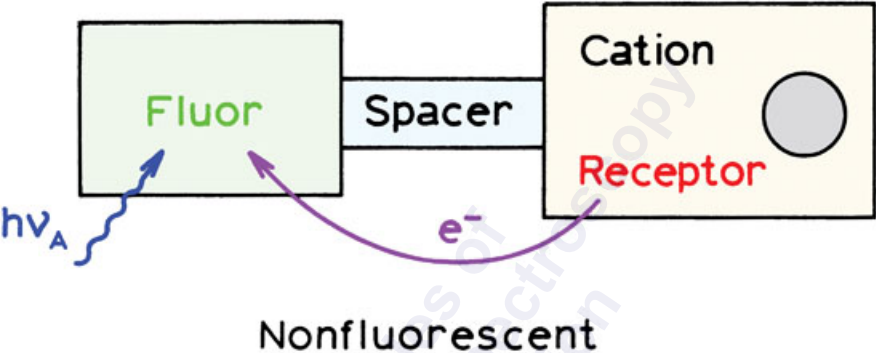
EMISSION INTENSITY



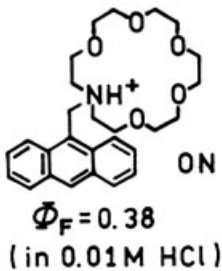
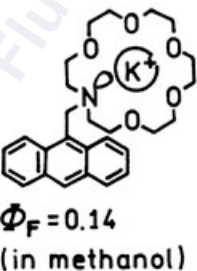
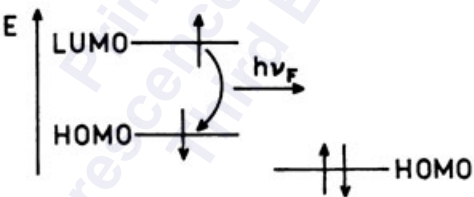
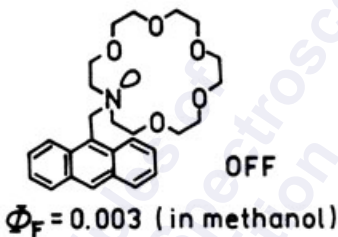
WAVELENGTH (nm)











FLUORESCENCE INTENSITY

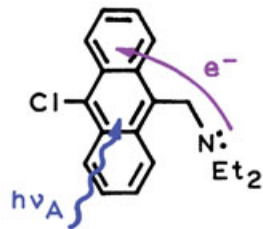
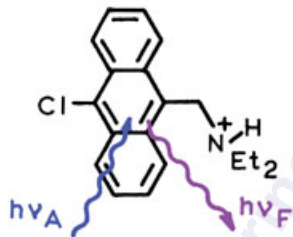
3.0

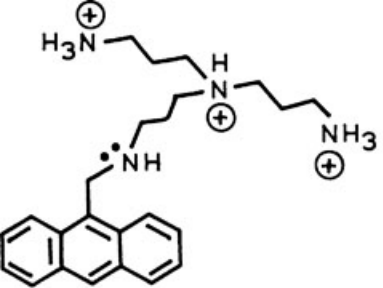
6.0

9.0

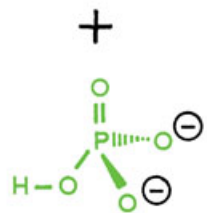
12.0

pH

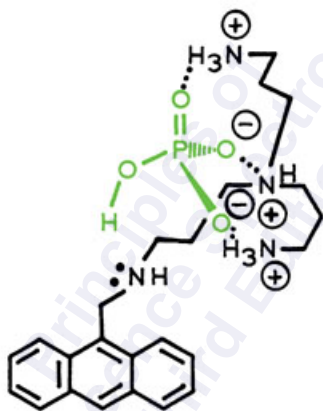
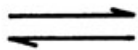




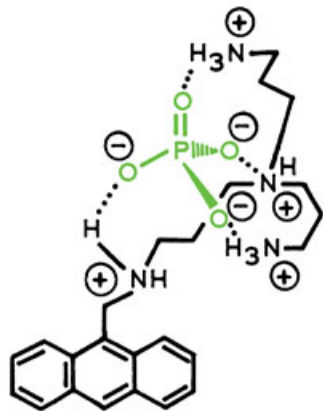
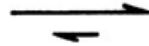
Low fluorescence



Nonfluorescent

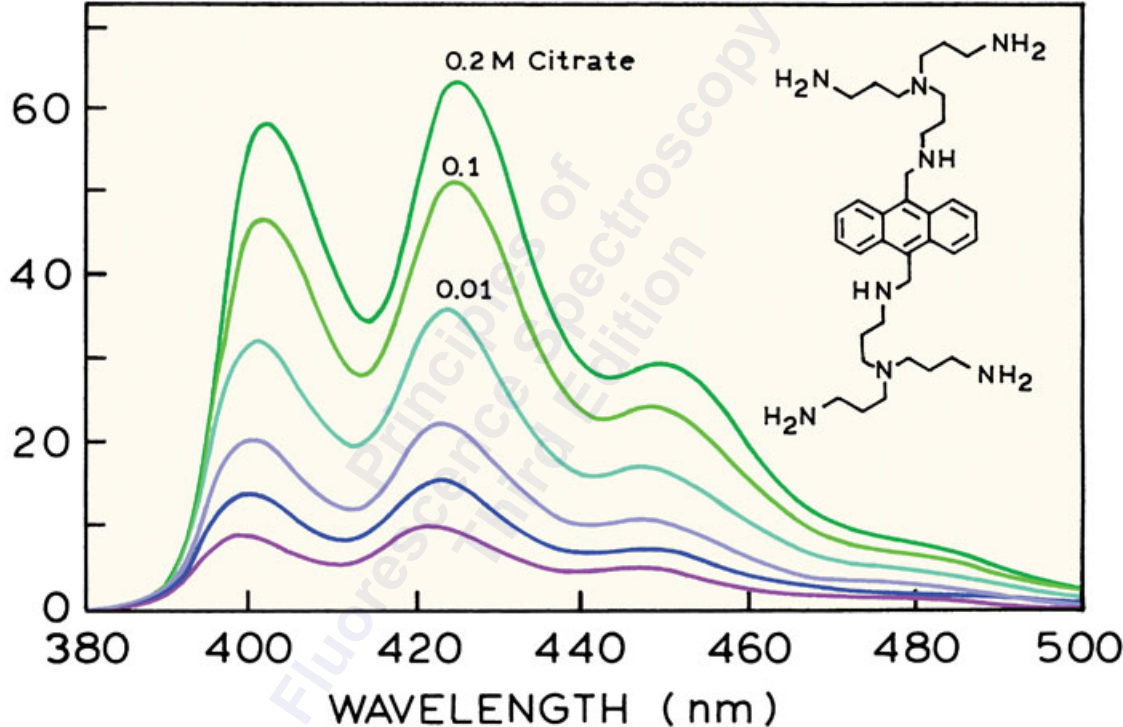


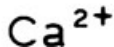
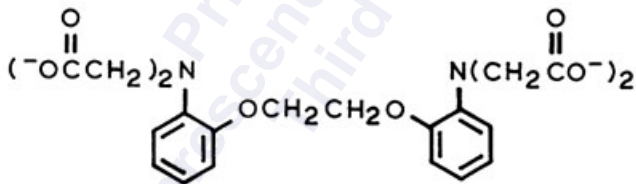
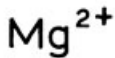
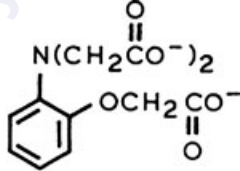
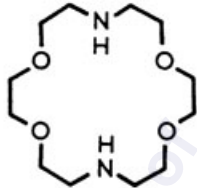
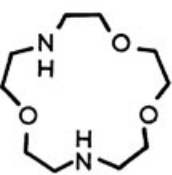
Low fluorescence

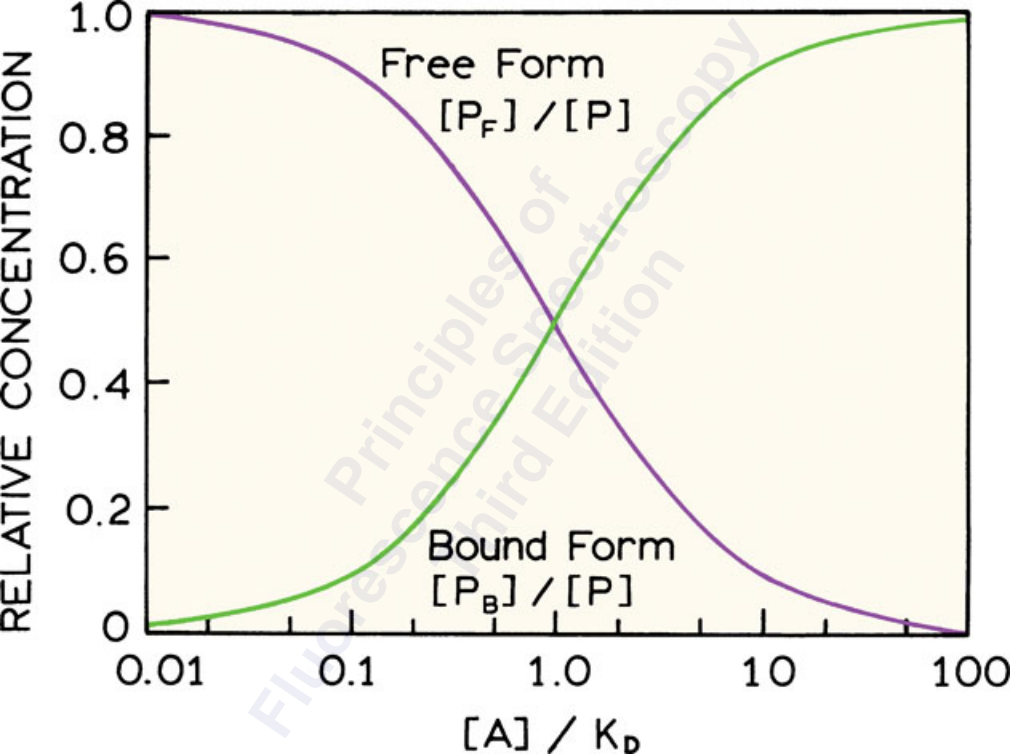


High fluorescence

FLUORESCENCE INTENSITY

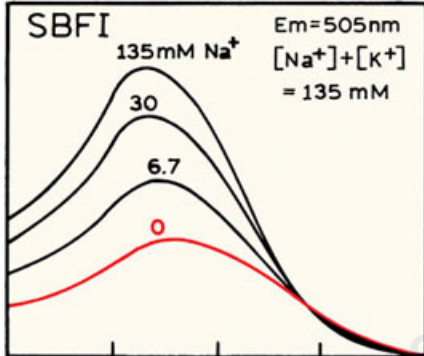






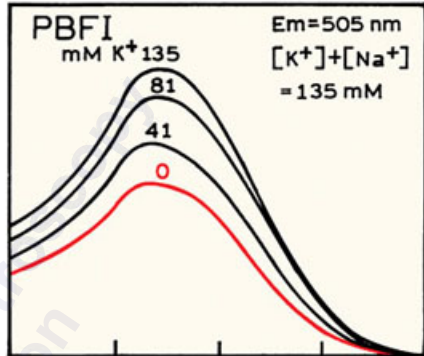


EXCITATION



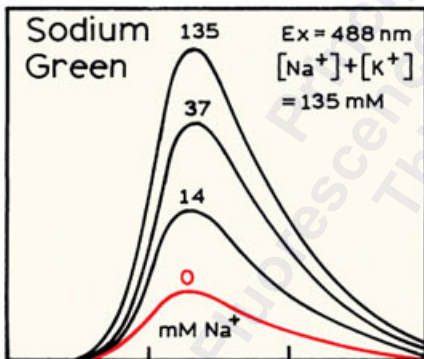
300 350 400

EXCITATION



300 350 400

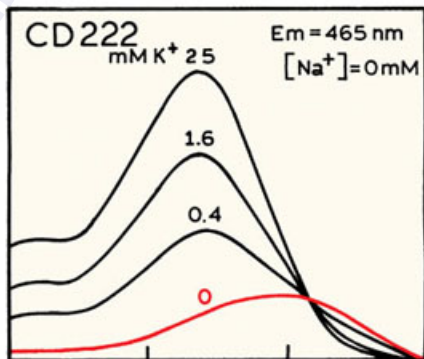
EMISSION



475 525 575 625

WAVELENGTH (nm)

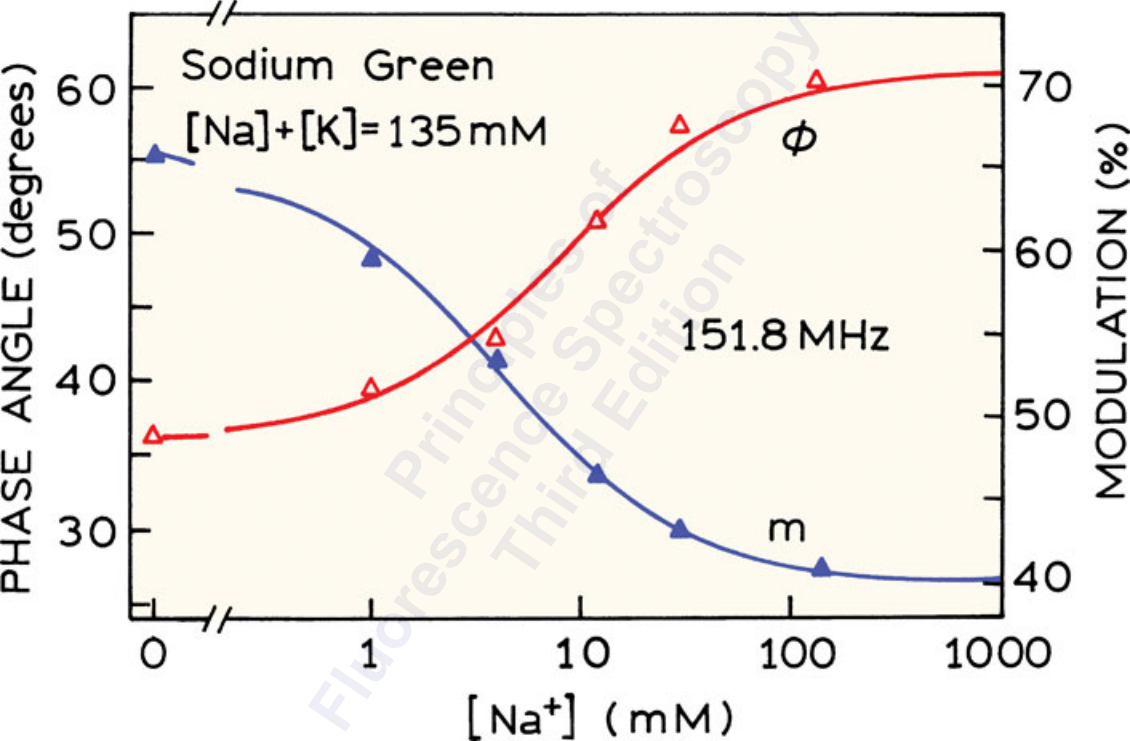
EXCITATION

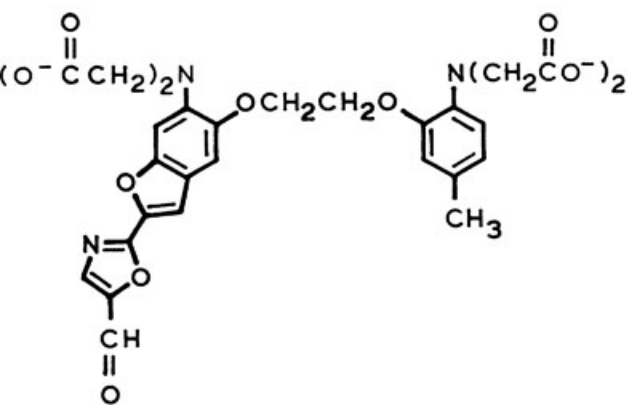


300 350 400 450

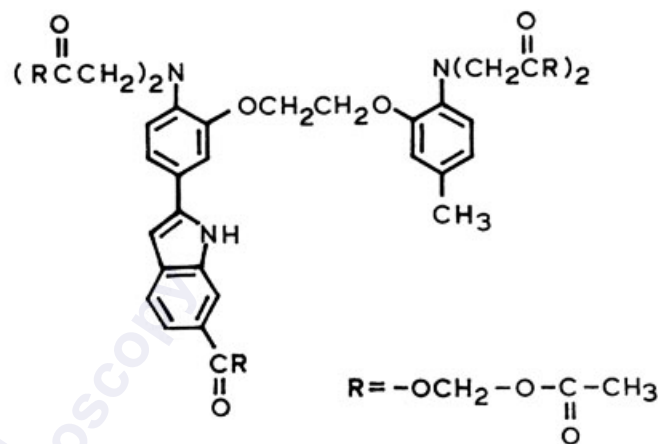
WAVELENGTH (nm)



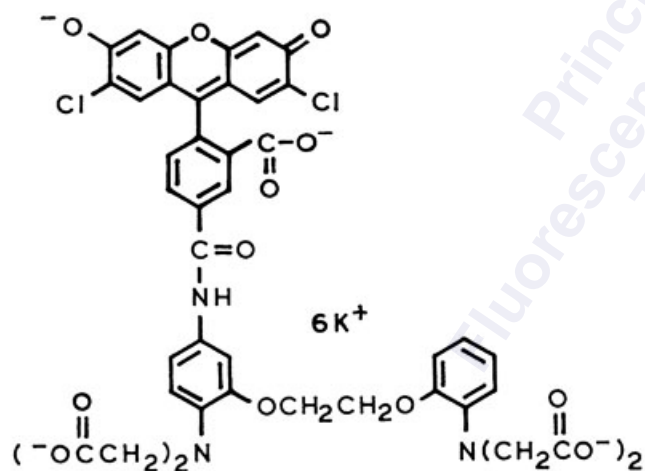




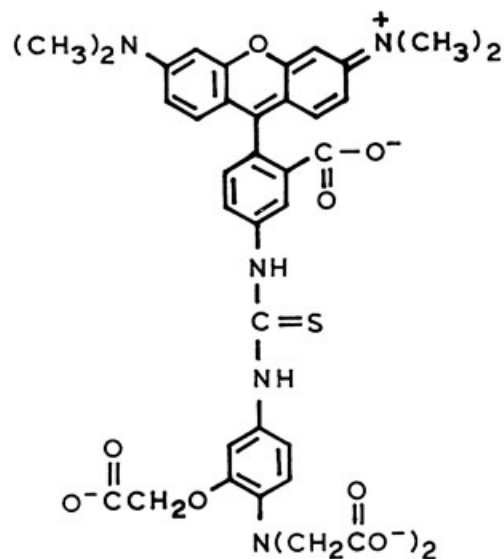
Fura-2,  $\text{Ca}^{2+}$



Indo-1, AM ester,  $\text{Ca}^{2+}$



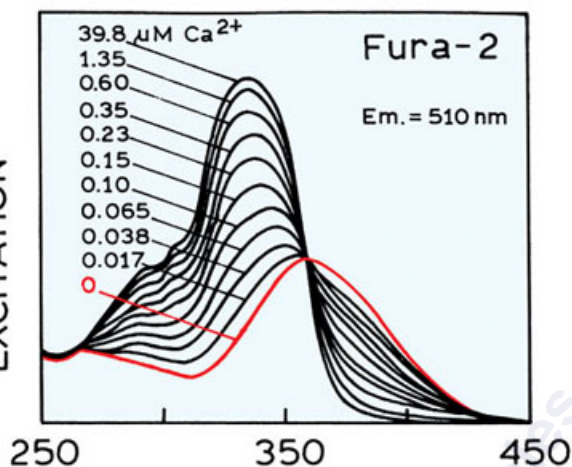
Calcium Green-1,  $\text{Ca}^{2+}$



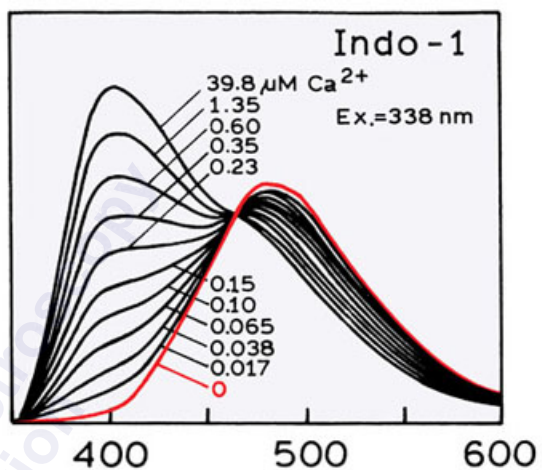
Magnesium Orange,  $\text{Mg}^{2+}$

FLUORESCENCE

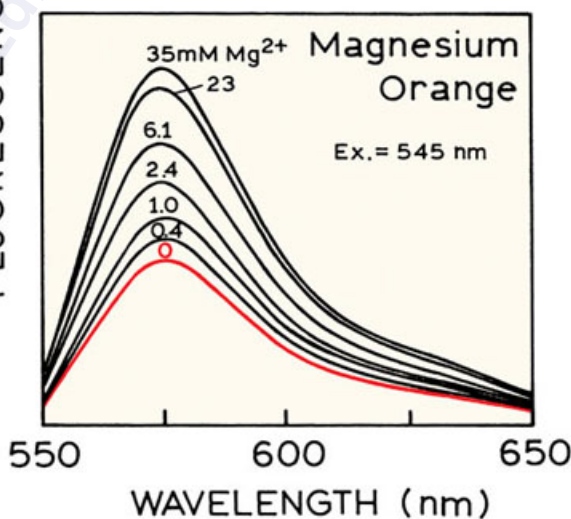
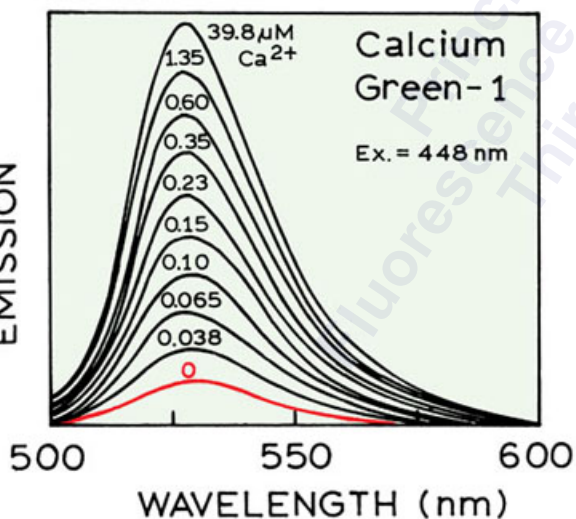
EXCITATION



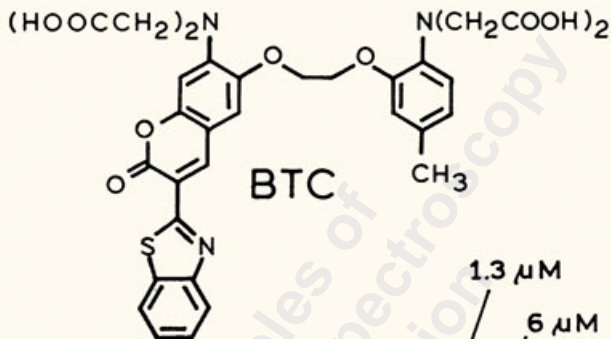
FLUORESCENCE EMISSION



EMISSION



FLUORESCENCE INTENSITY



Emission 540 nm

1.3  $\mu\text{M}$

6  $\mu\text{M}$

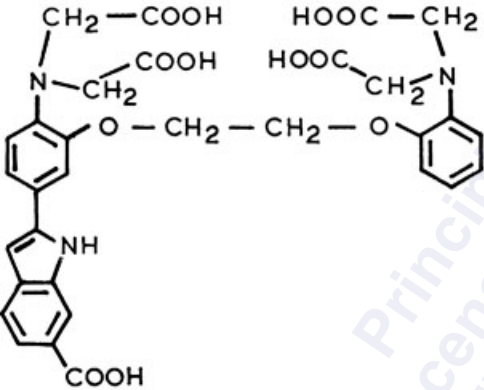
10  $\mu\text{M}$

20  $\mu\text{M}$

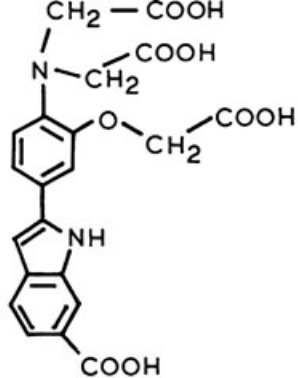
100  $\mu\text{M}$

50  $\mu\text{M}$

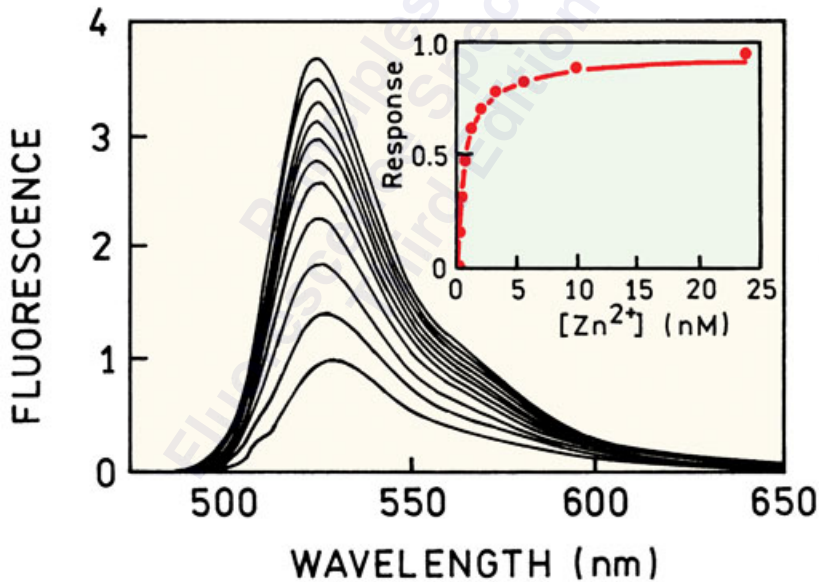
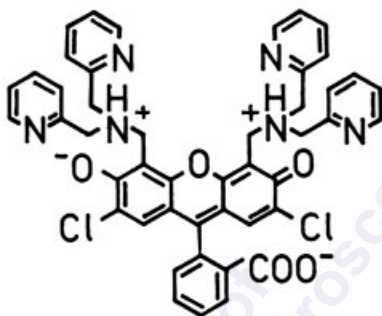
EXCITATION WAVELENGTH (nm)



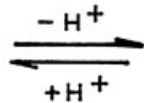
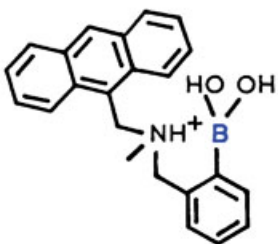
INDO-1



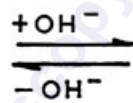
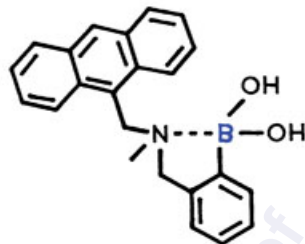
Mag-INDO-1



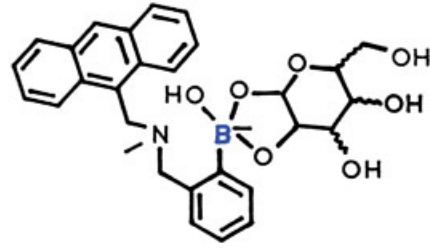
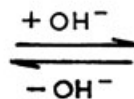
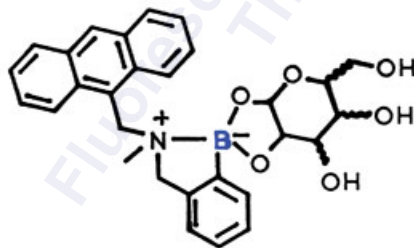
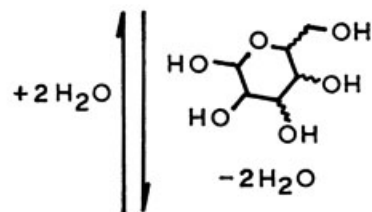
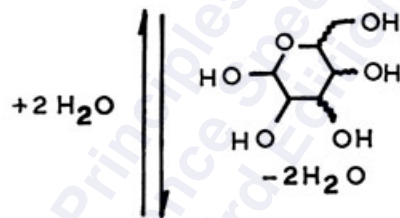
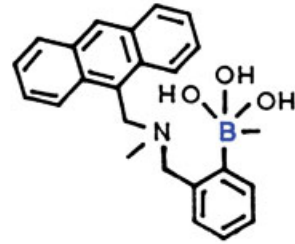
Fluorescent



Nonfluorescent

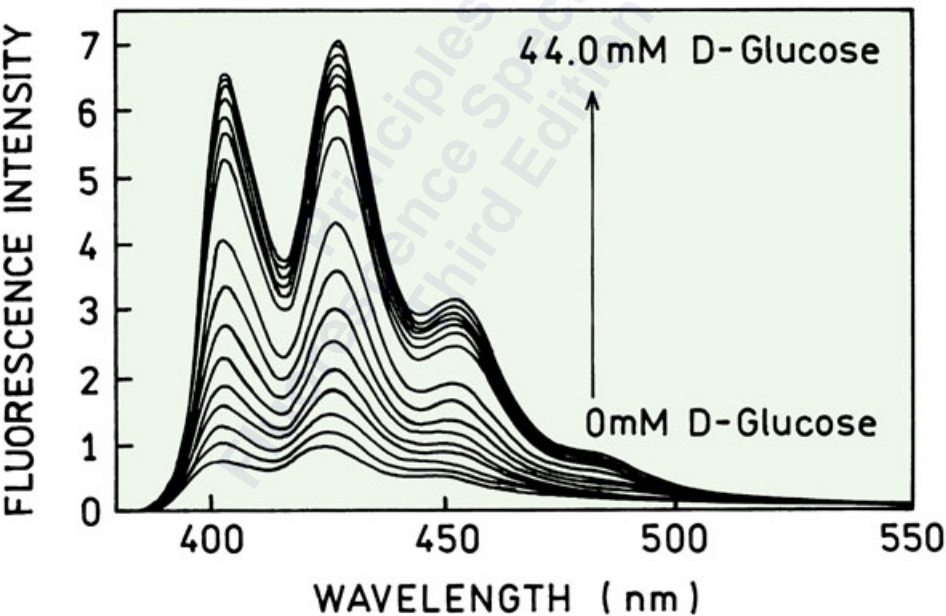
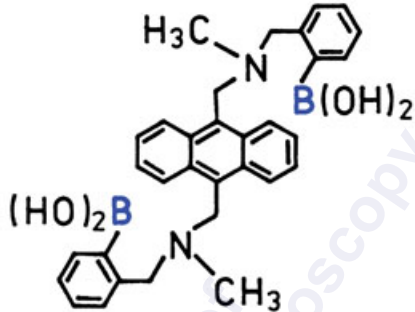


Nonfluorescent

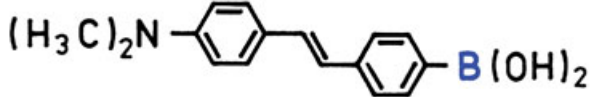


Fluorescent

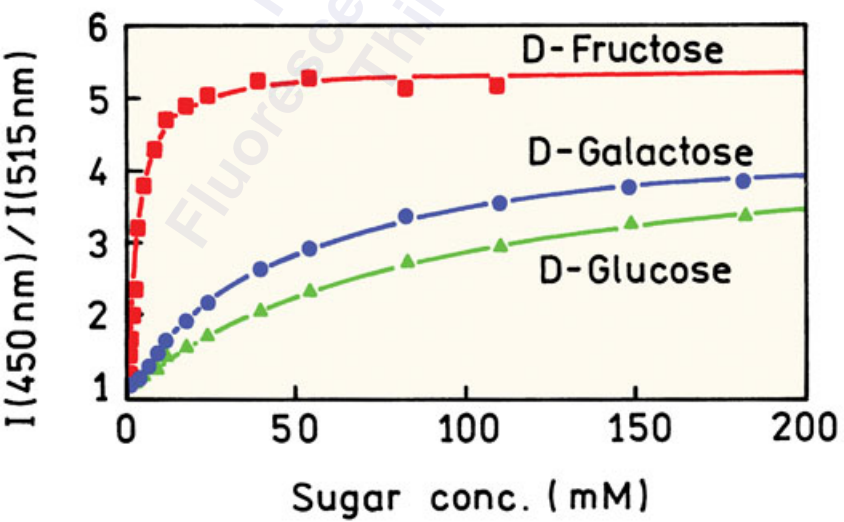
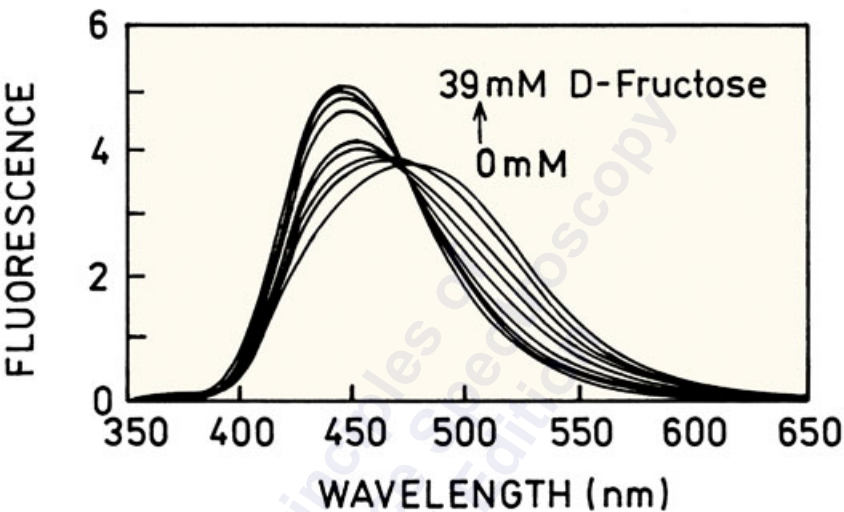
Nonfluorescent

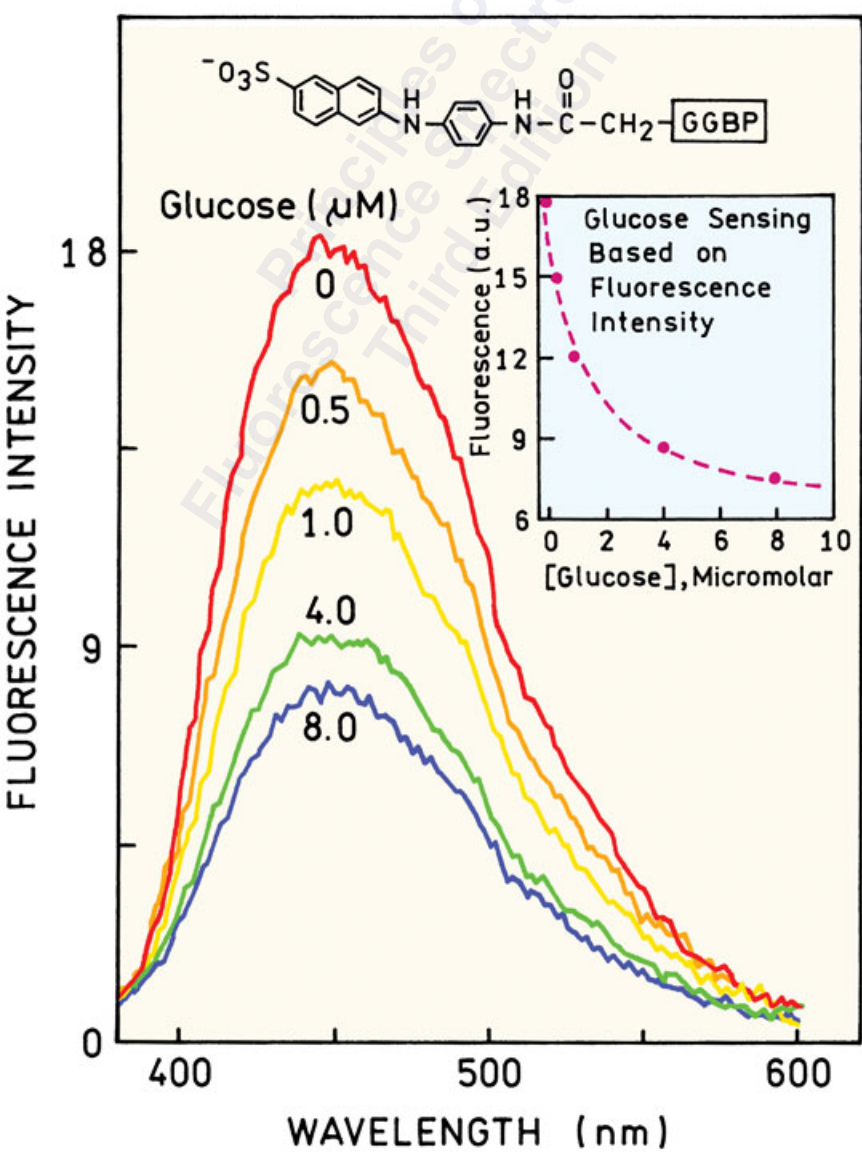
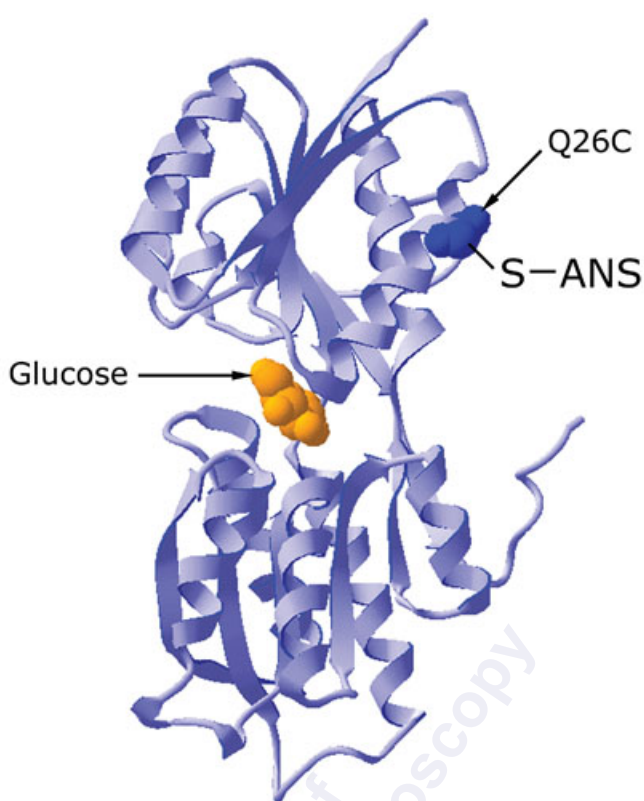




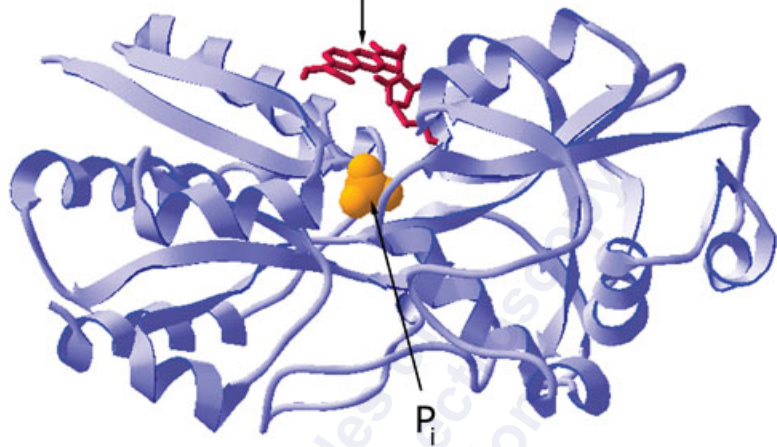


DSTBA

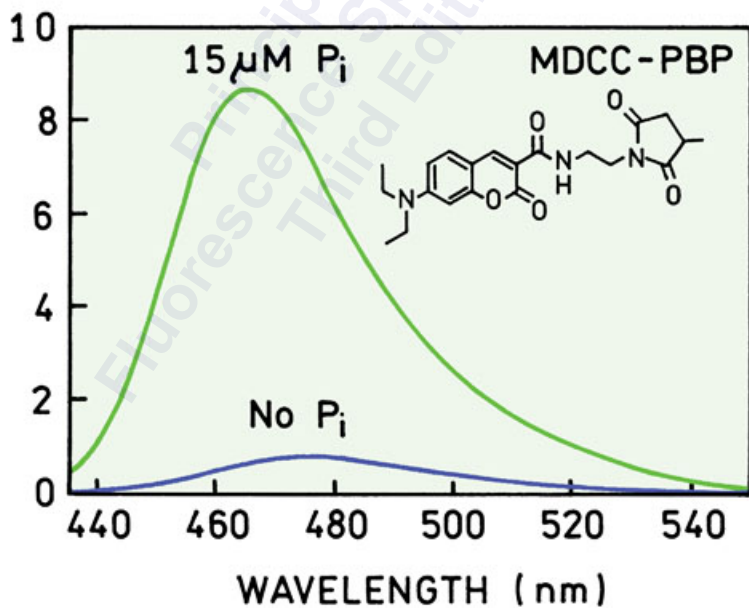


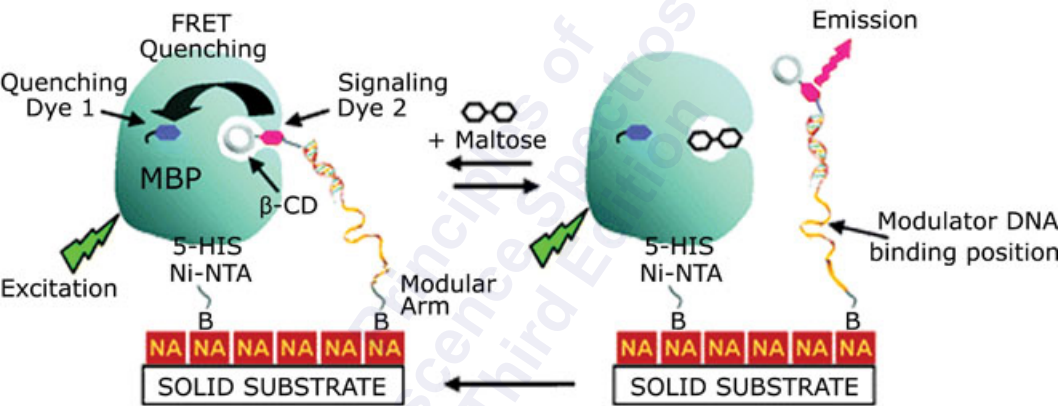


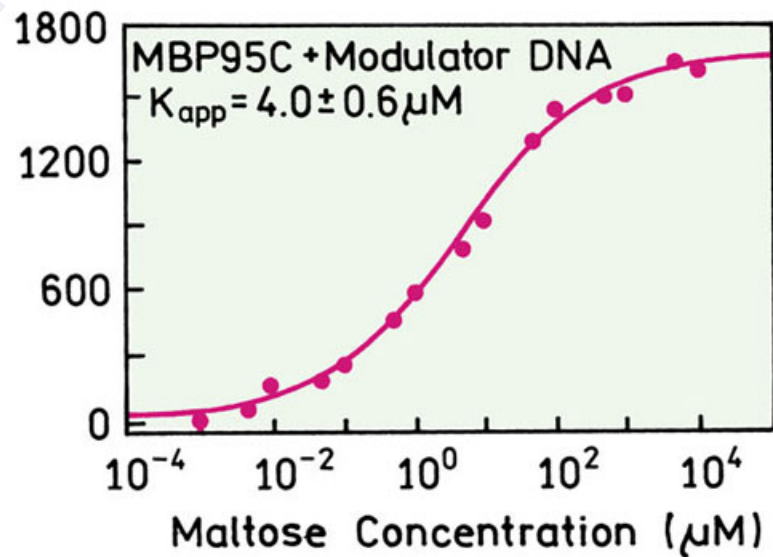
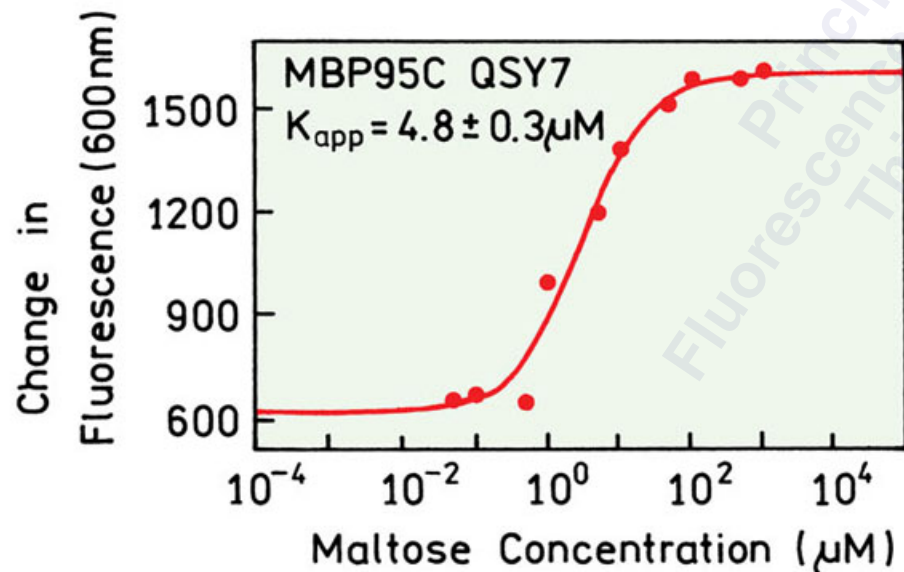
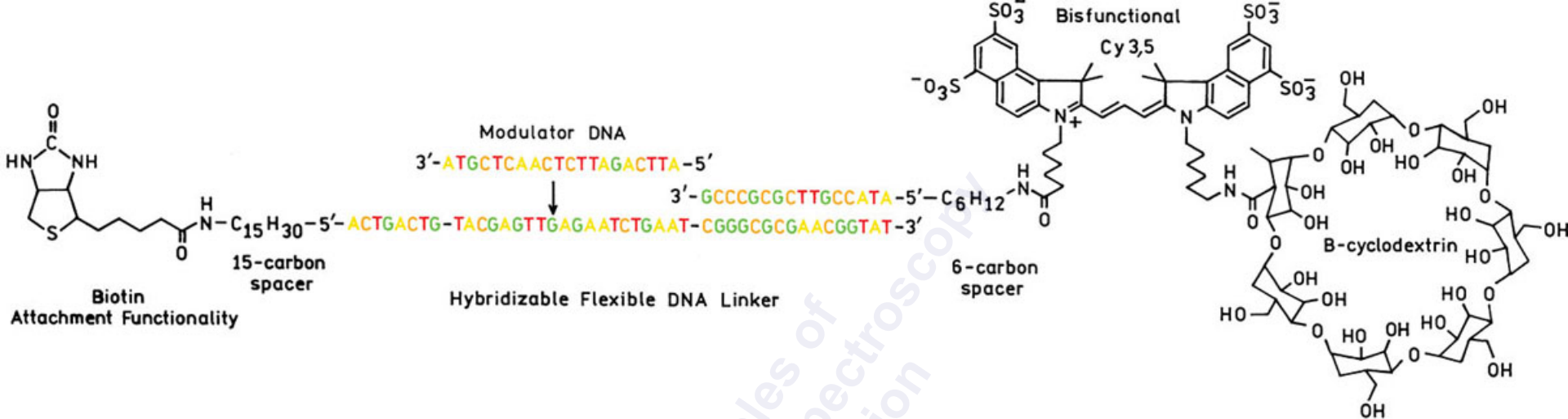
MDCC



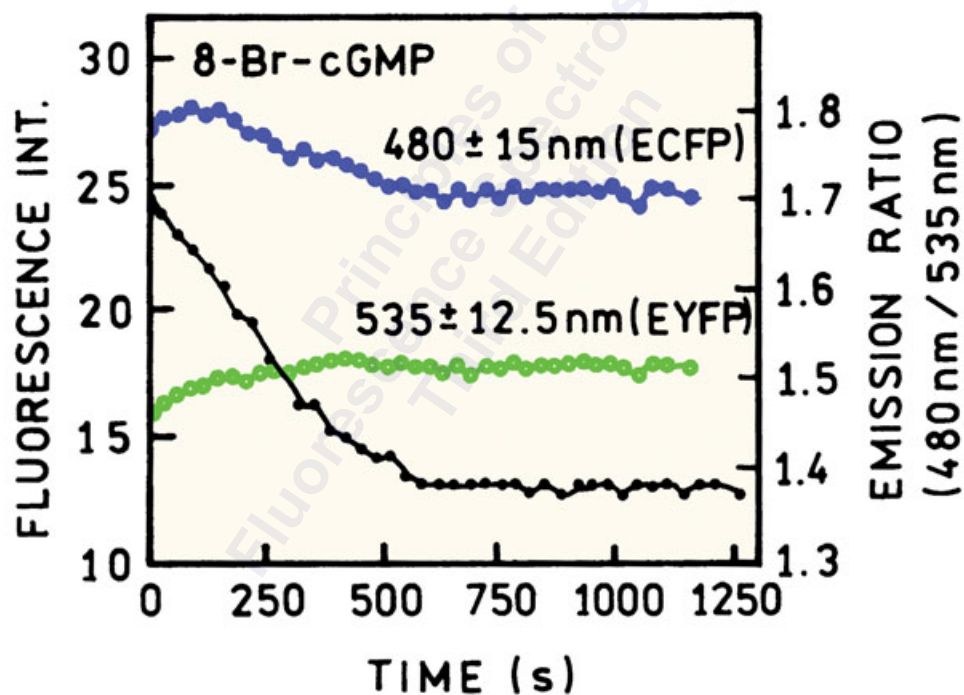
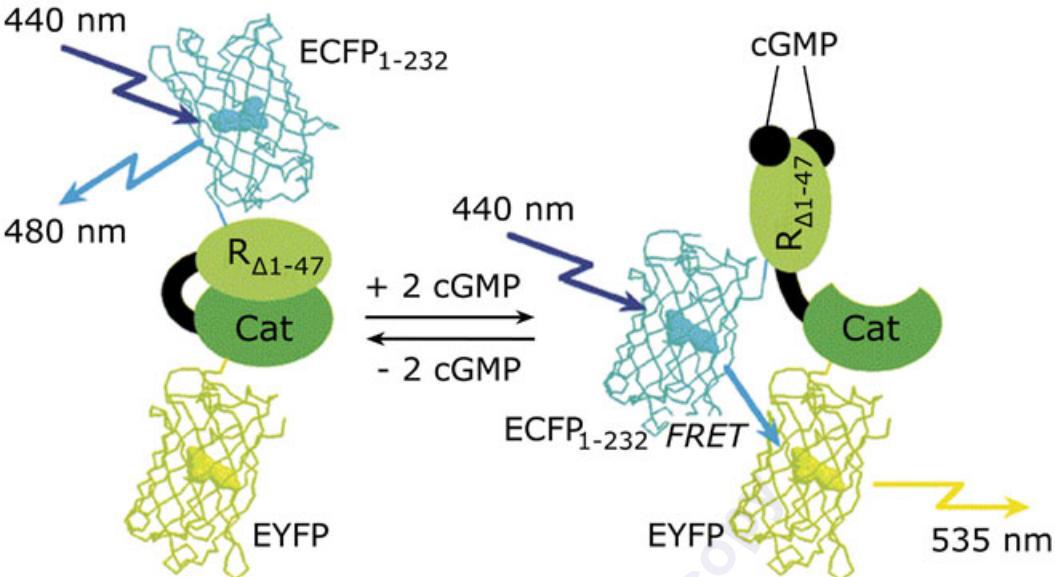
FLUORESCENCE







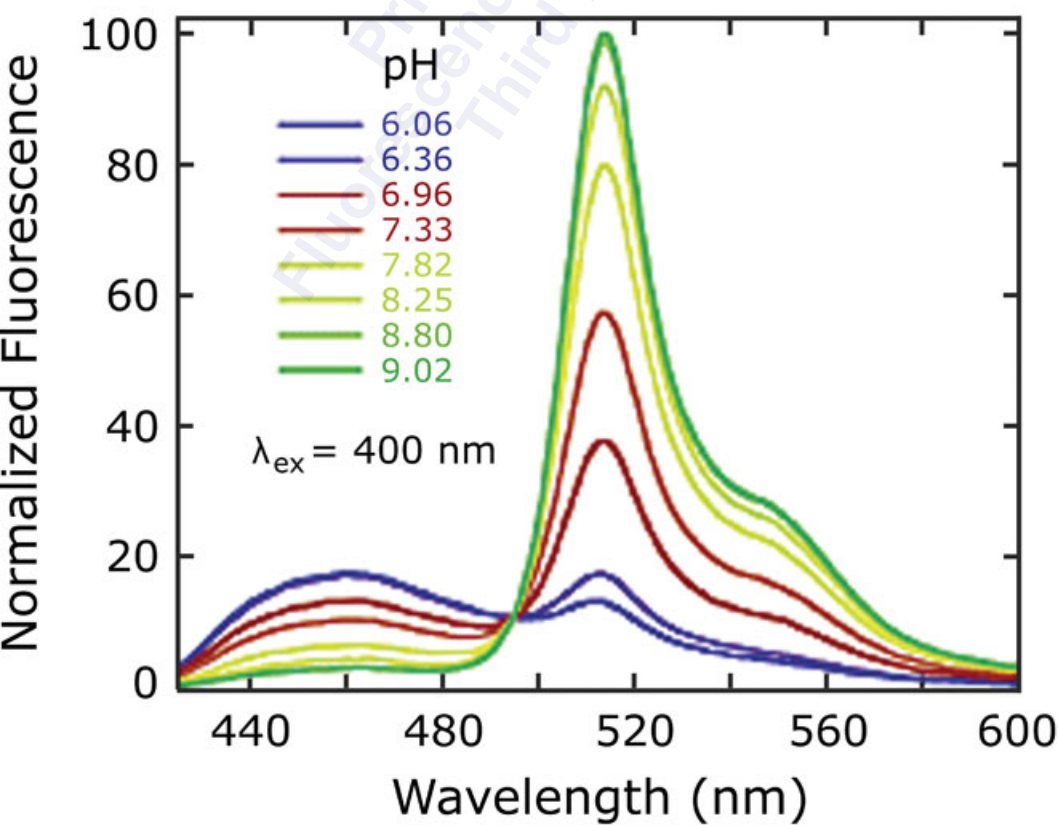
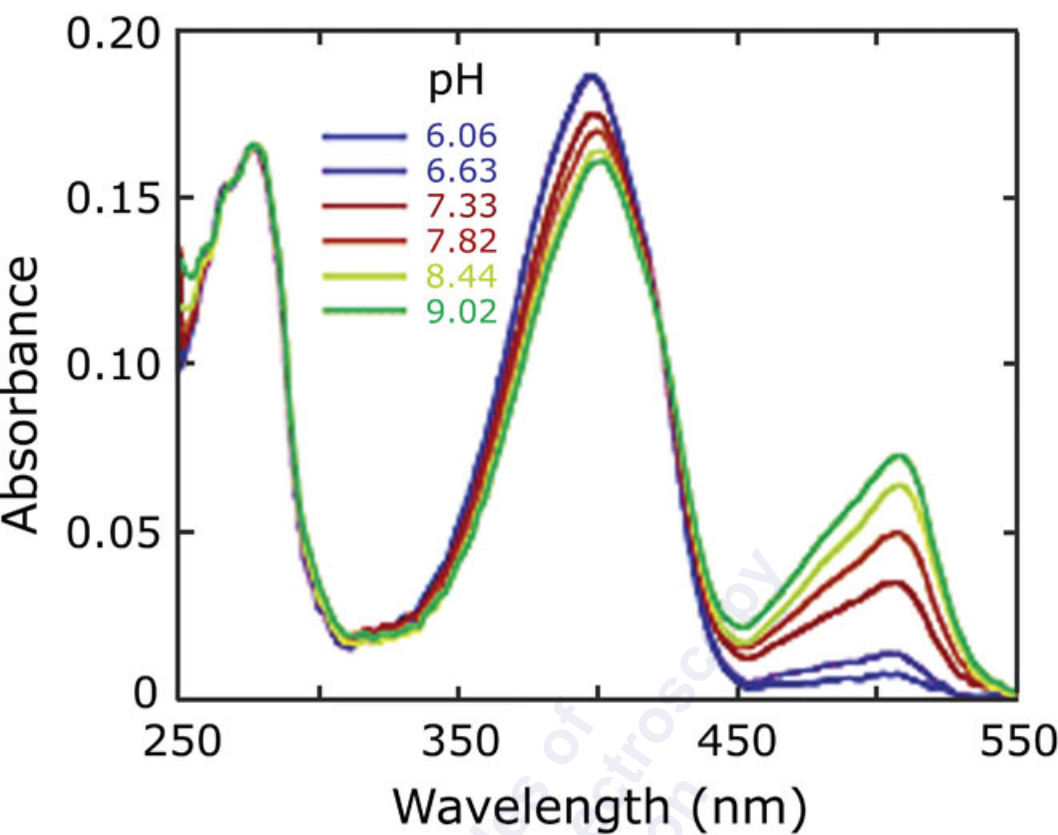


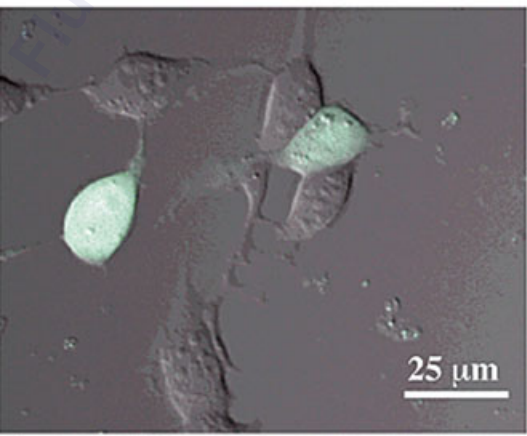
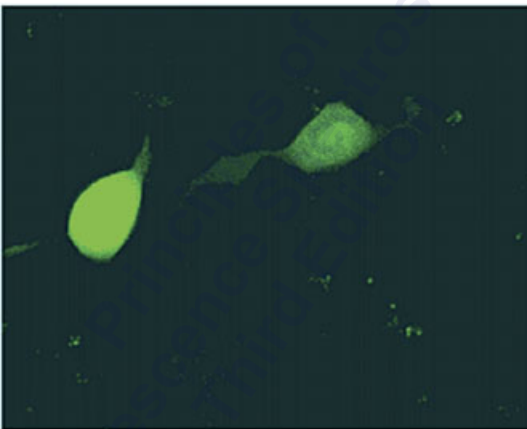
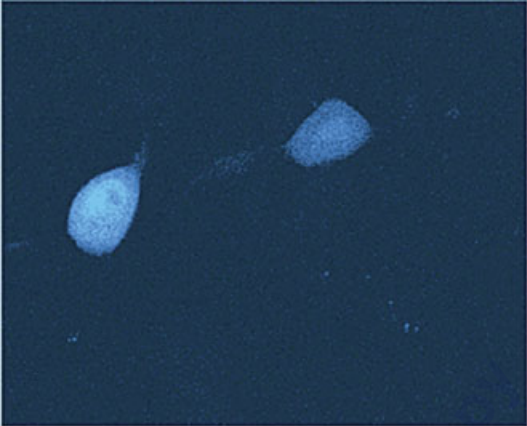


Del 1

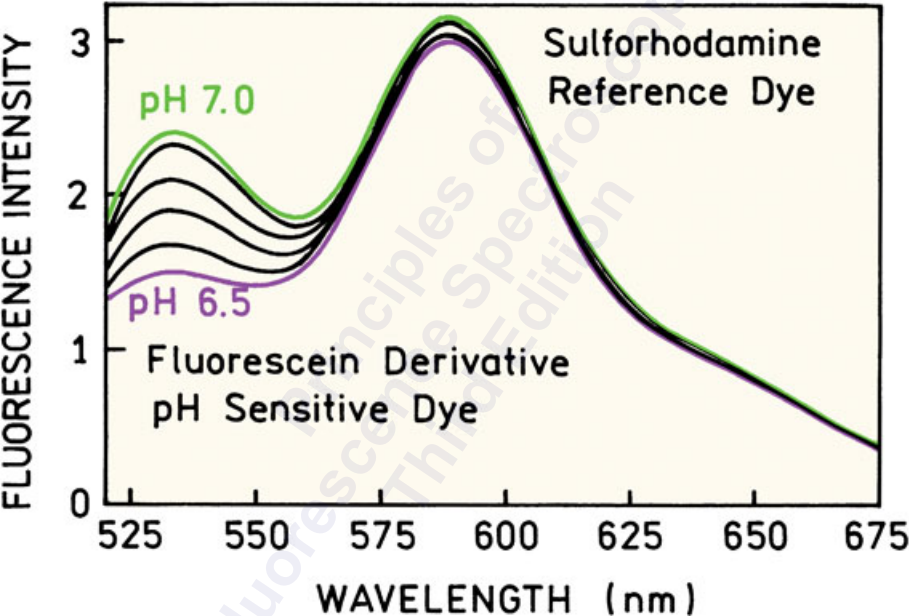
Emission ratio 480/535

0.7 1.8



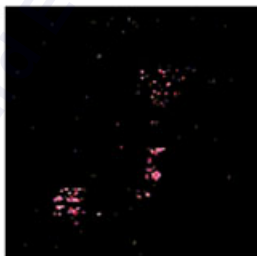
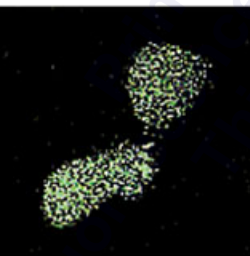
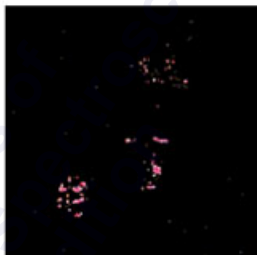
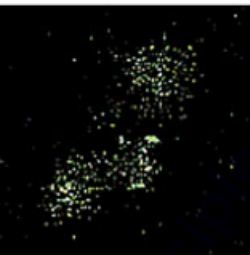






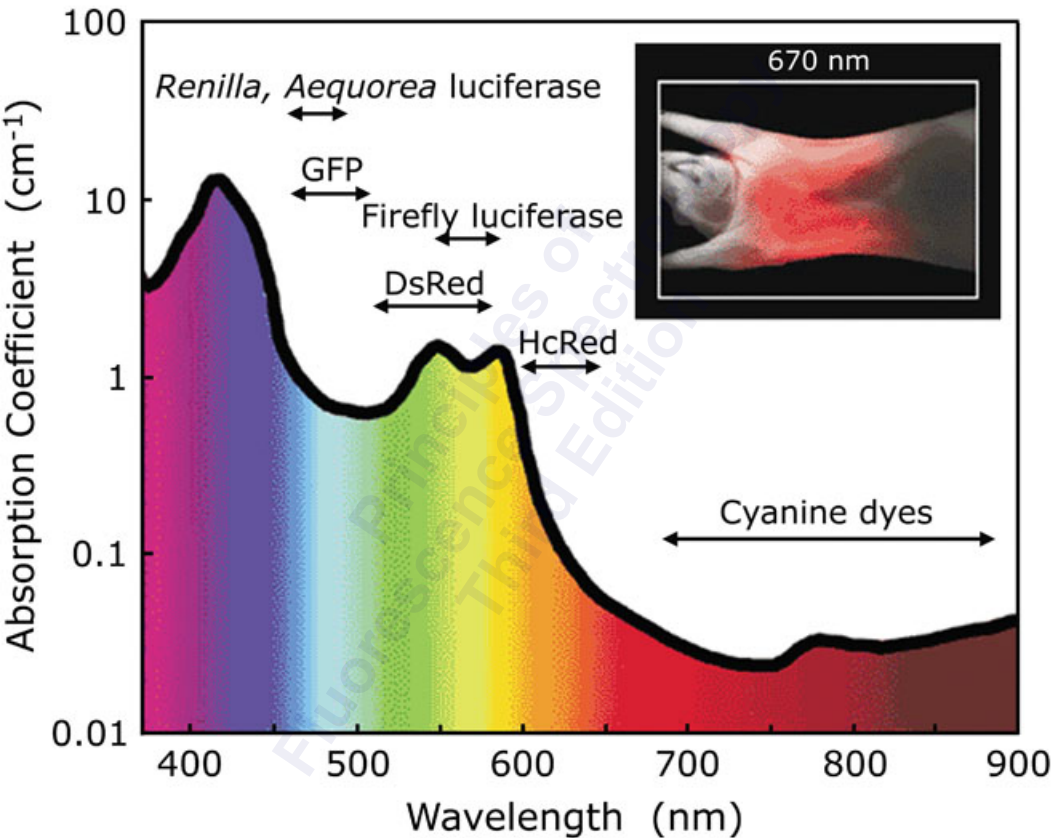
Calcium Green

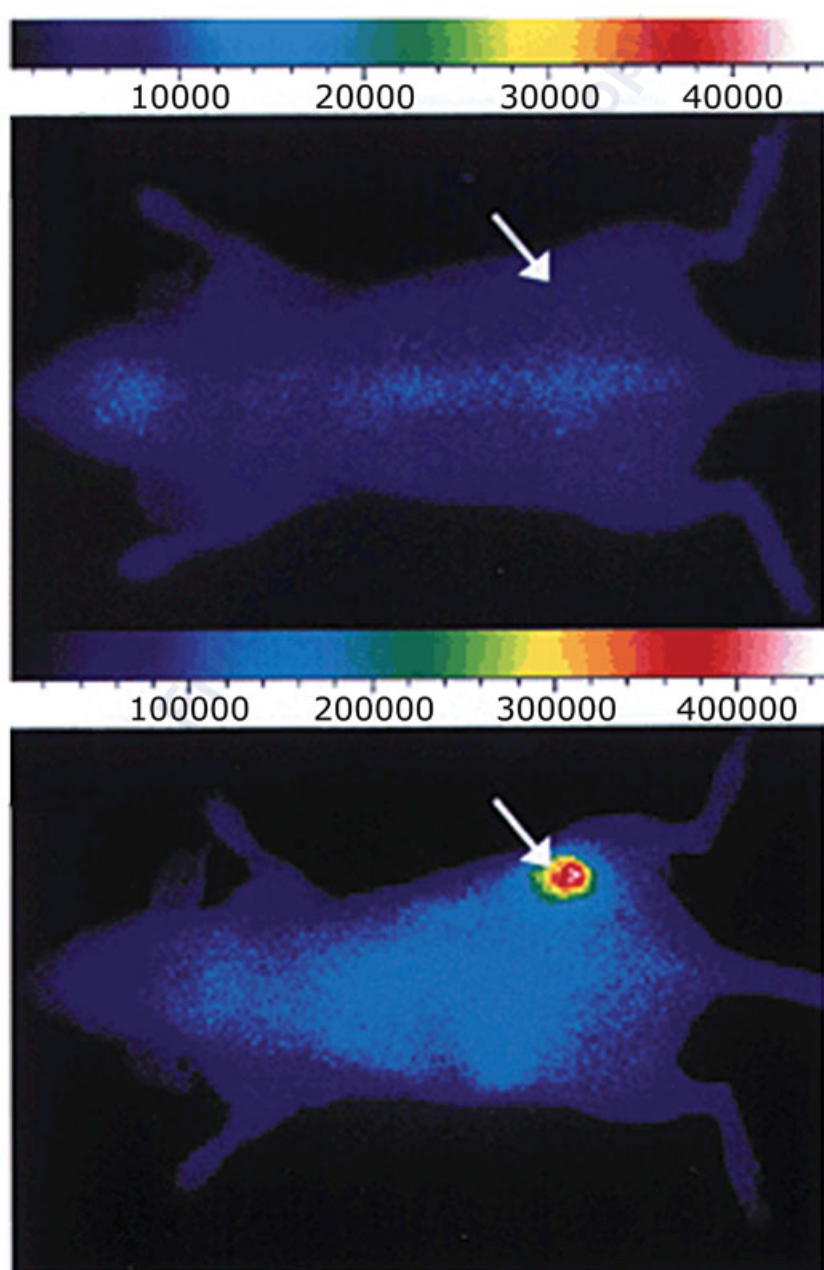
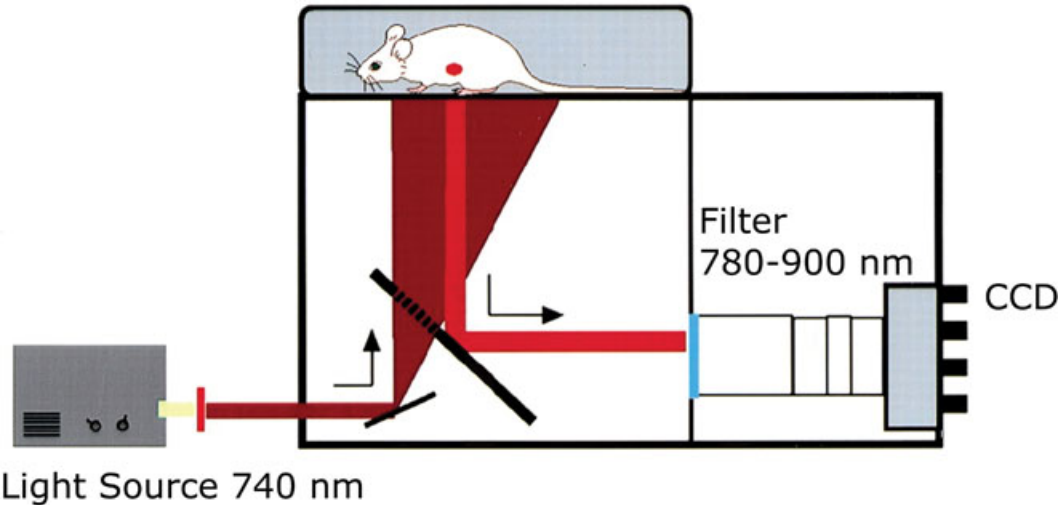
Sulforhodamine

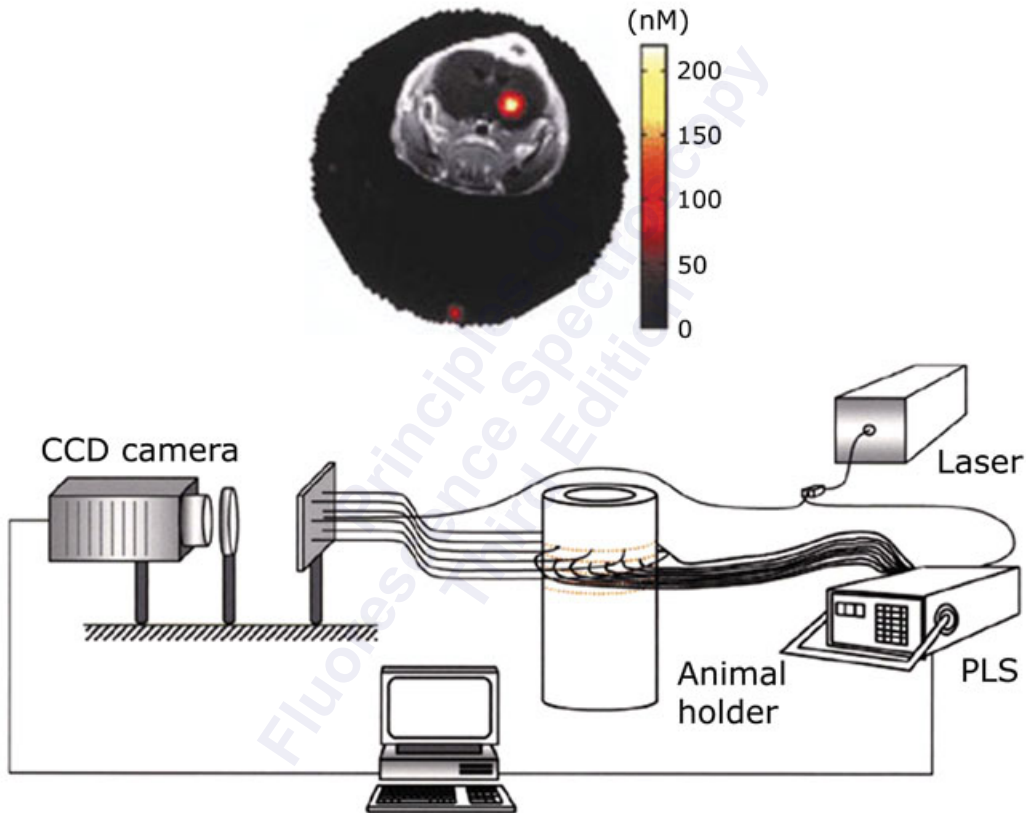


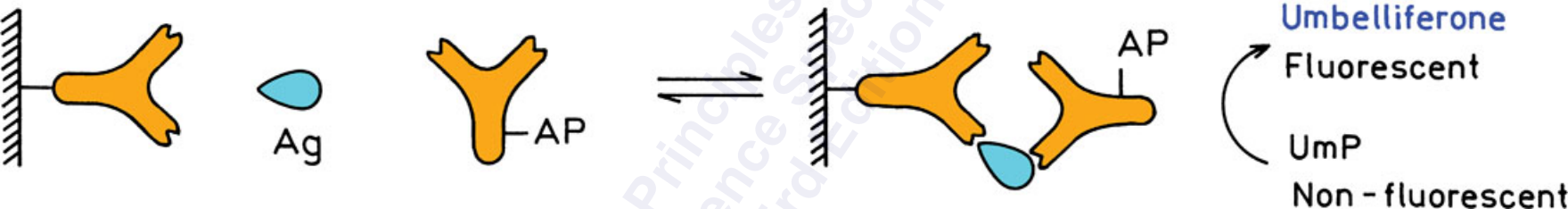
500 nm

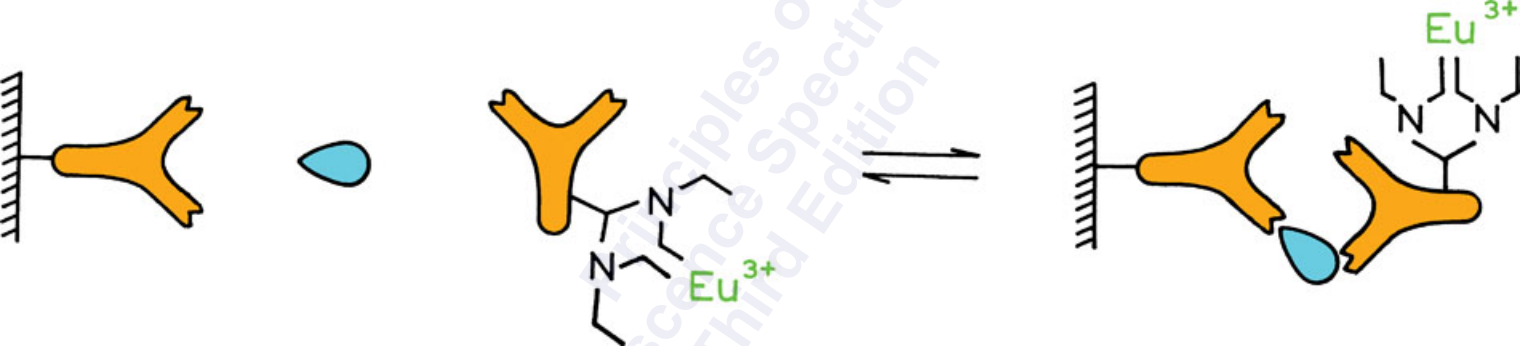
590 nm

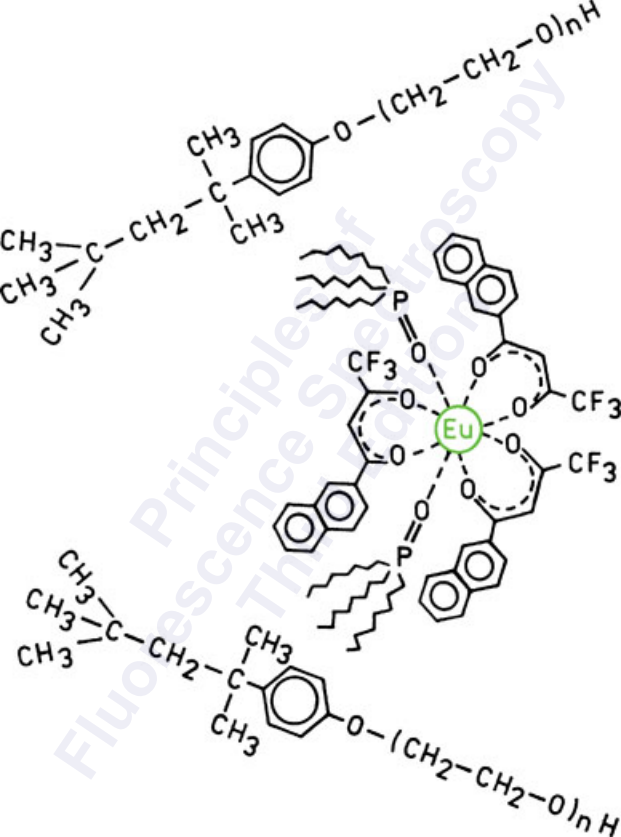




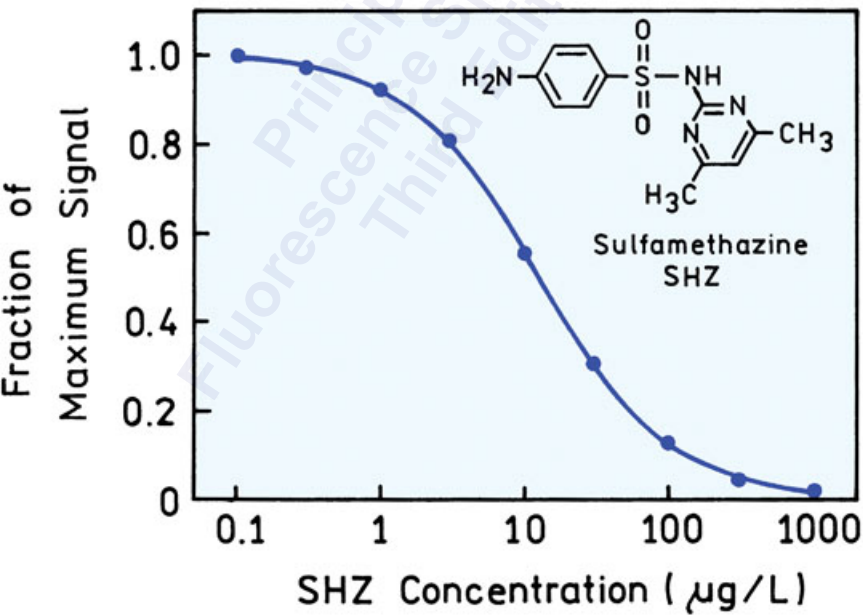
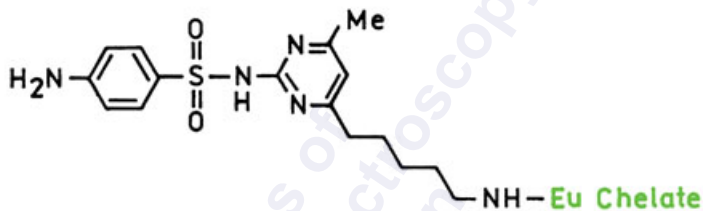
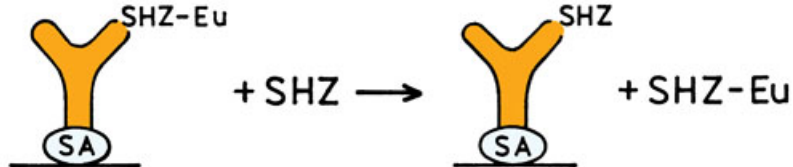


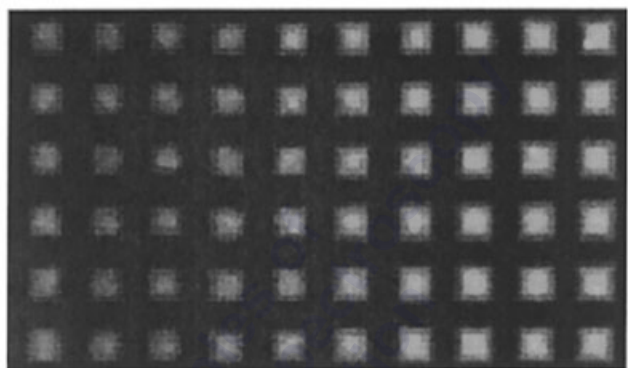
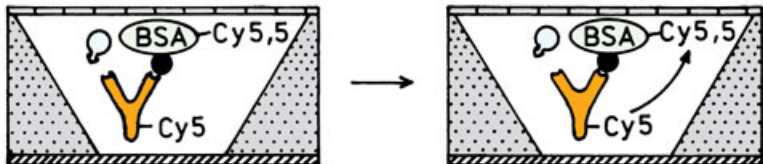






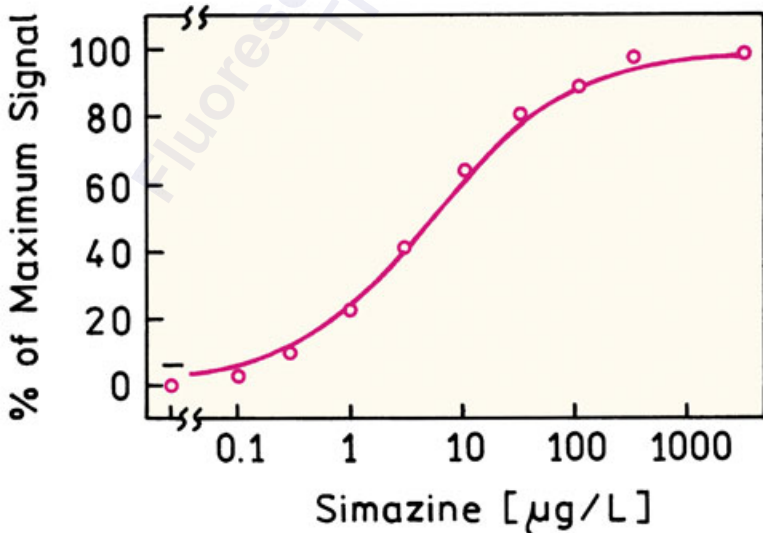


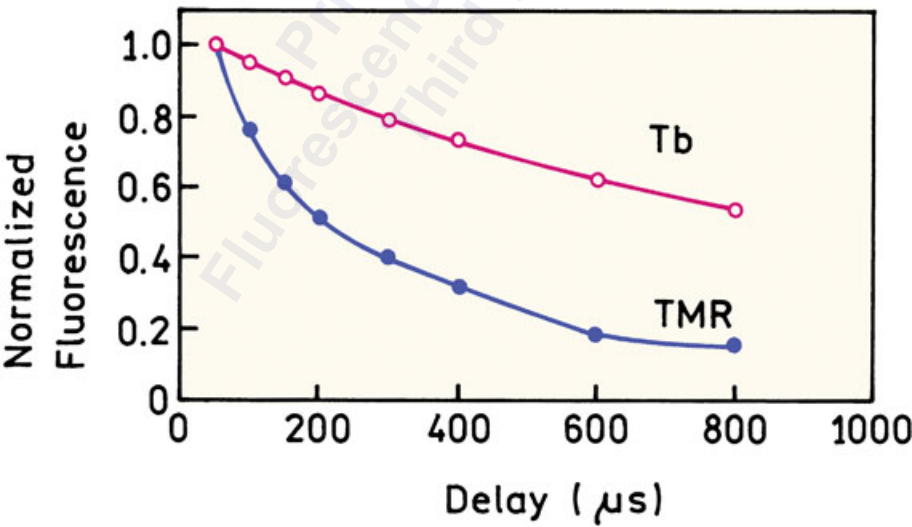
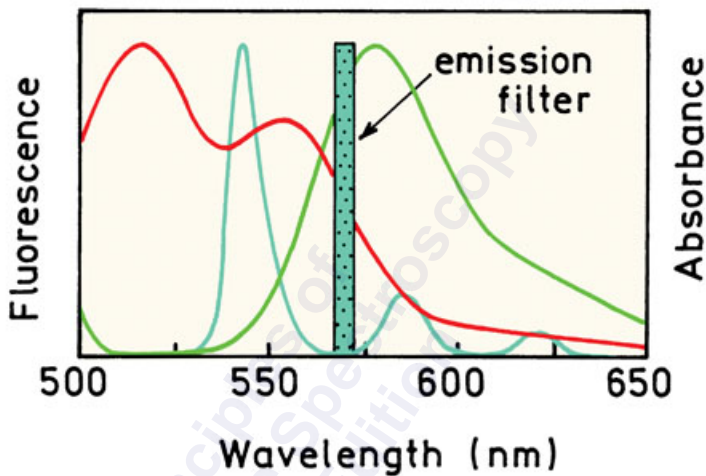
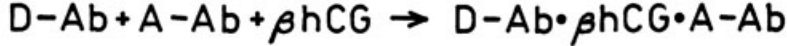


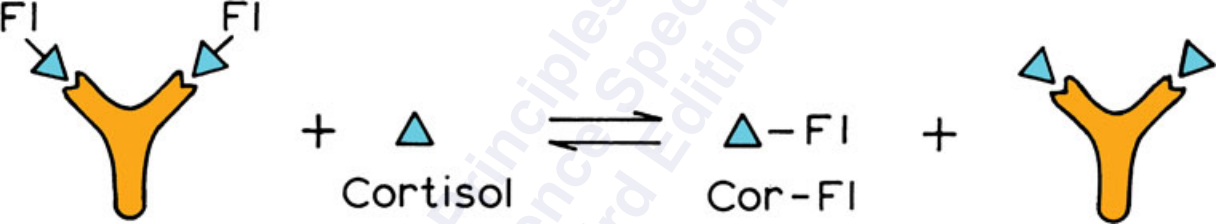


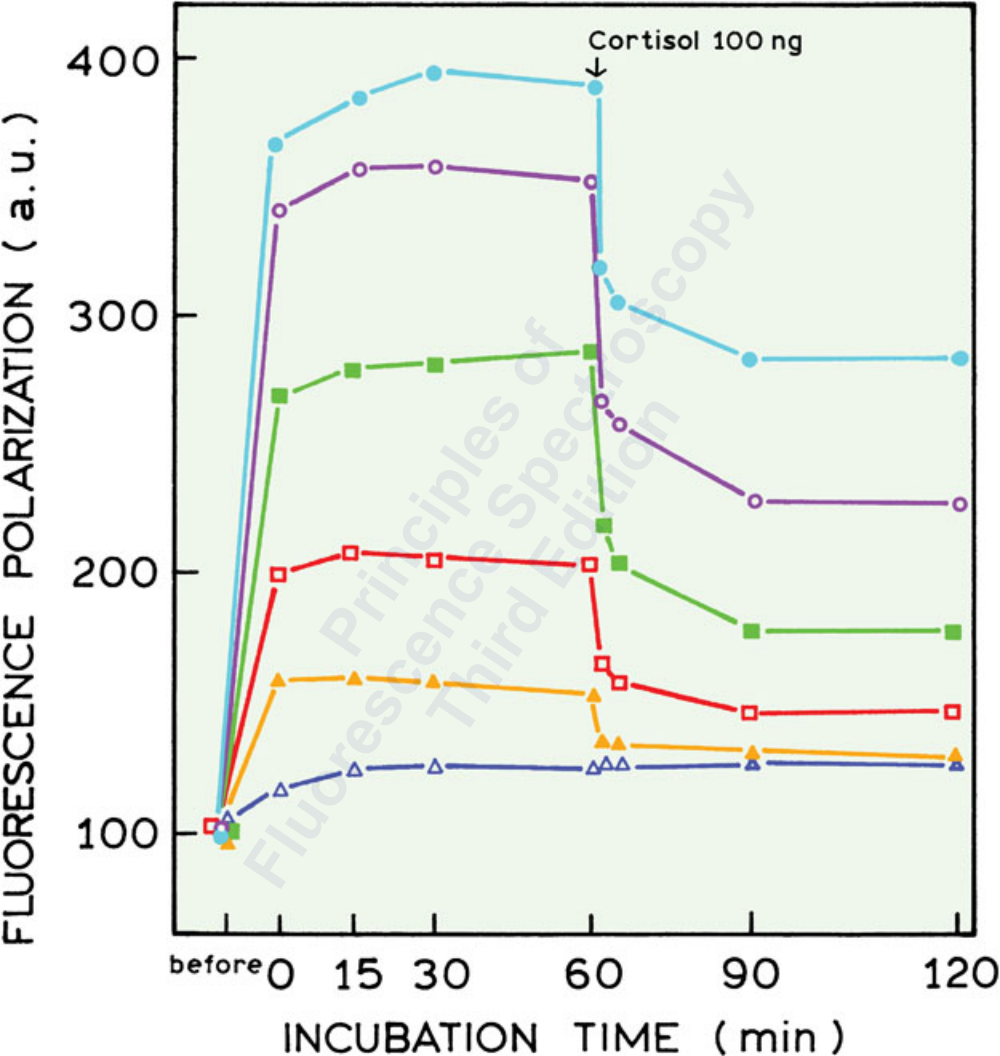
0 0.1 0.3 1 3 10 30 100 300 1000

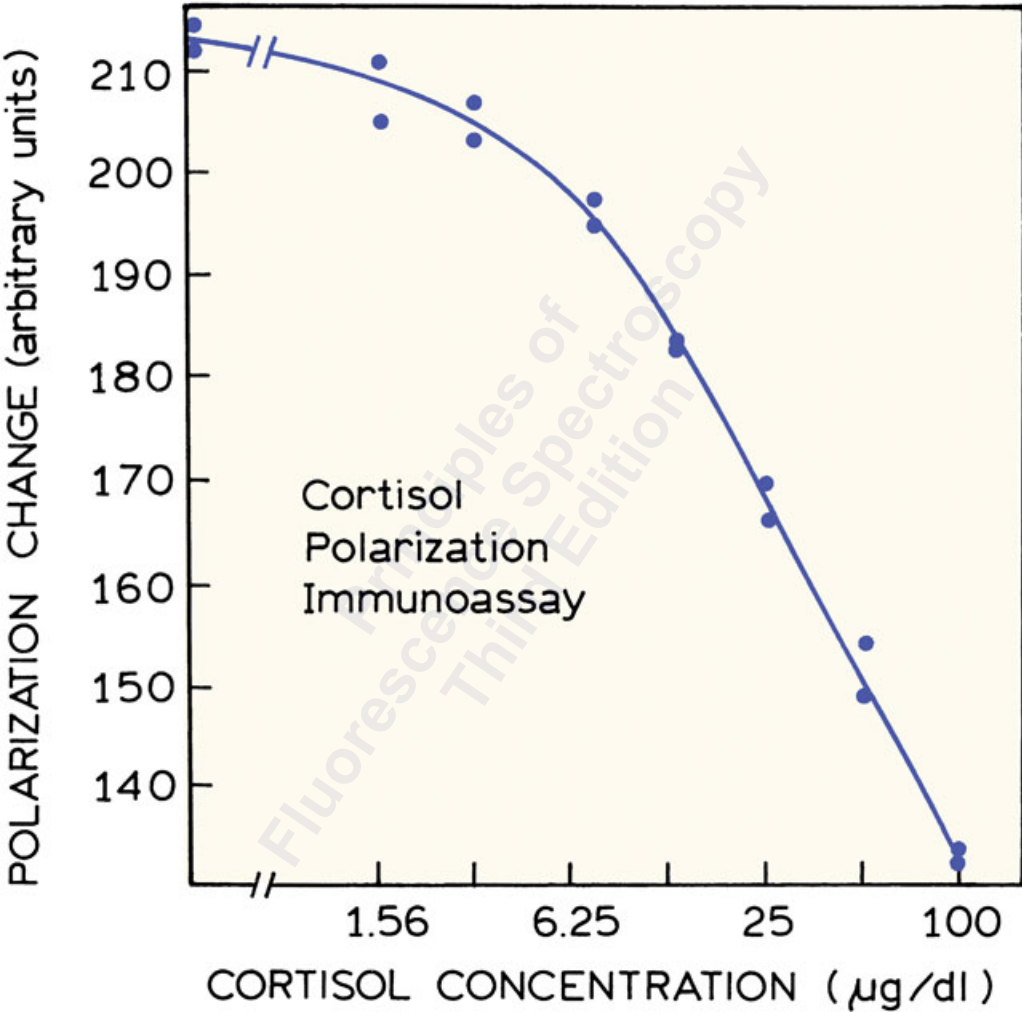
Simazine [ $\mu\text{g/L}$ ]

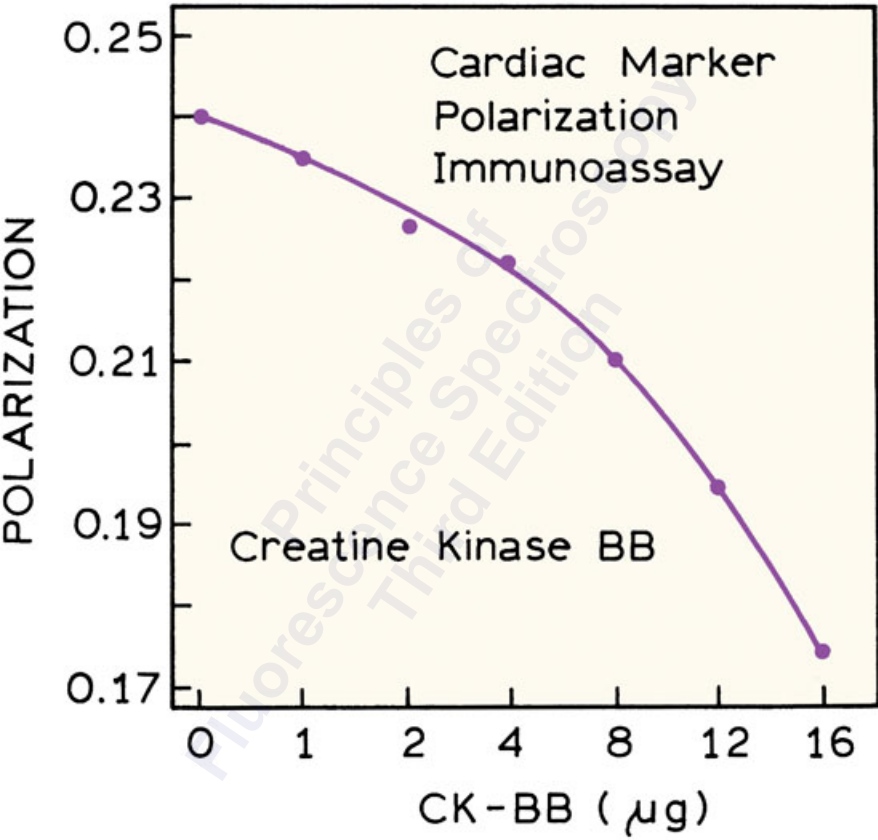




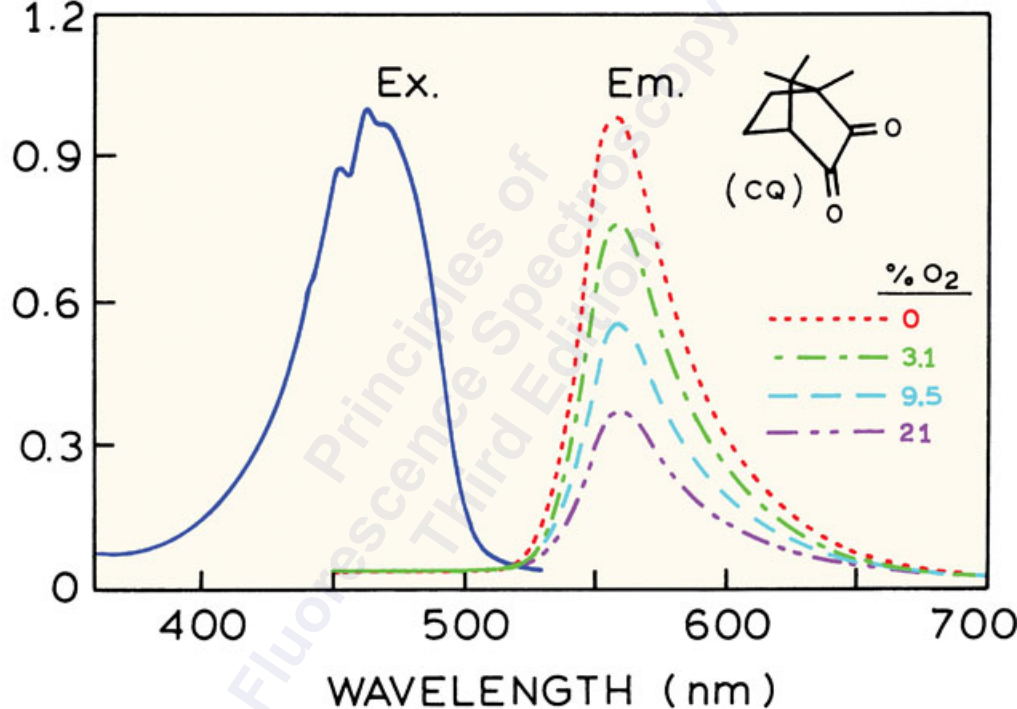




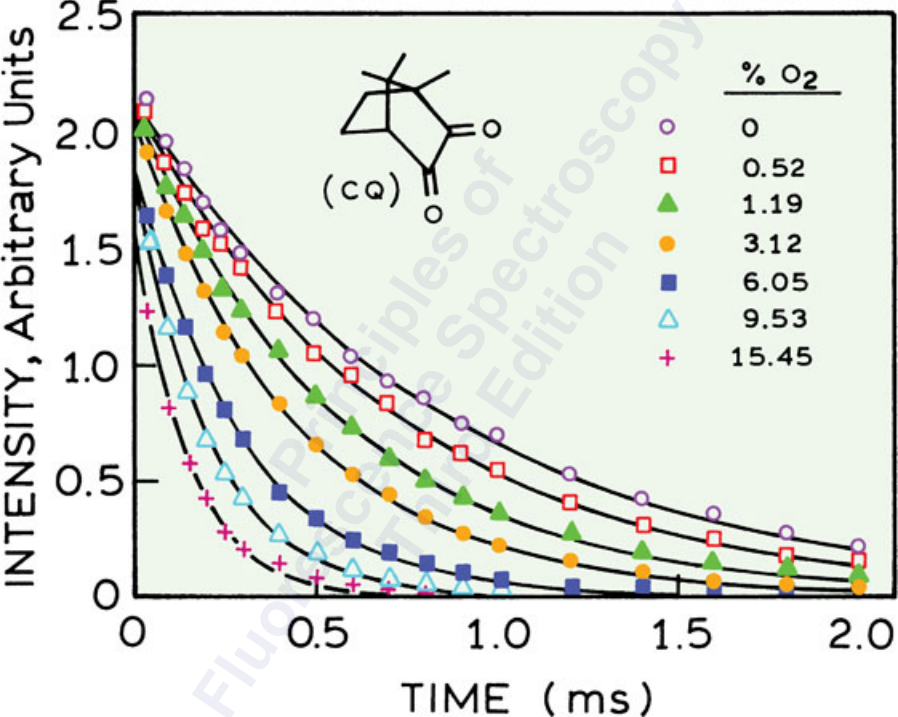


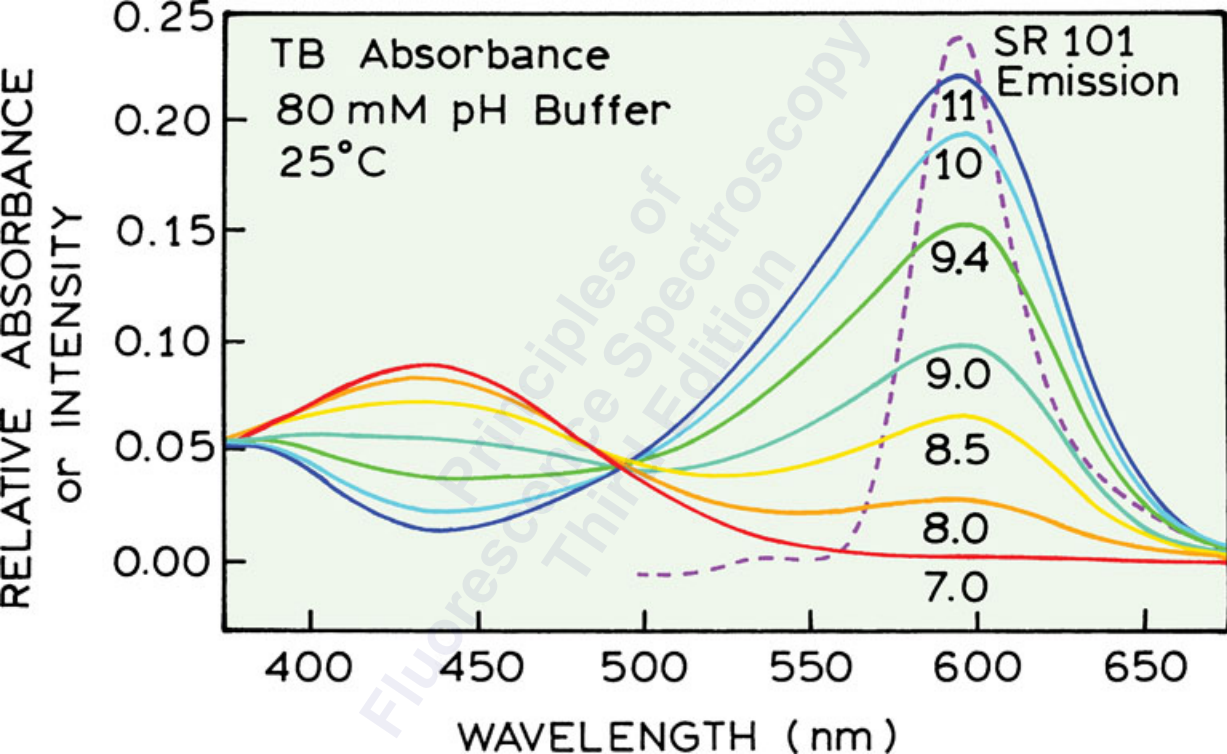


INTENSITY, NORMALIZED



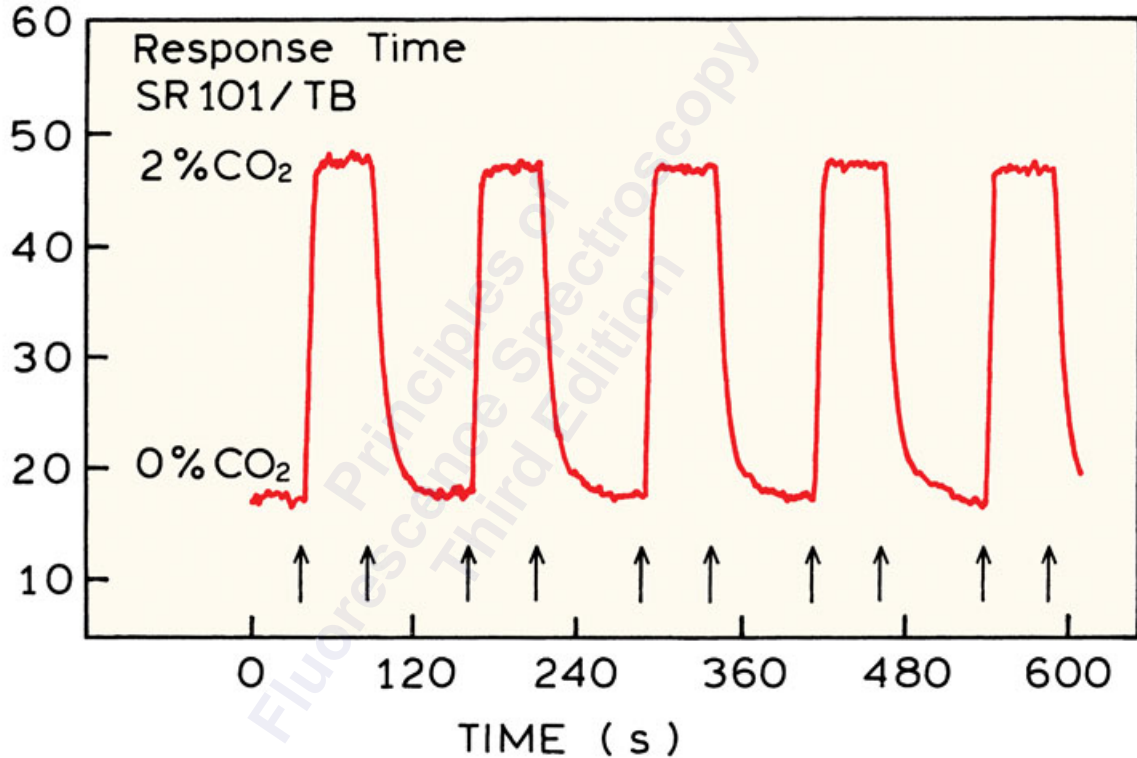


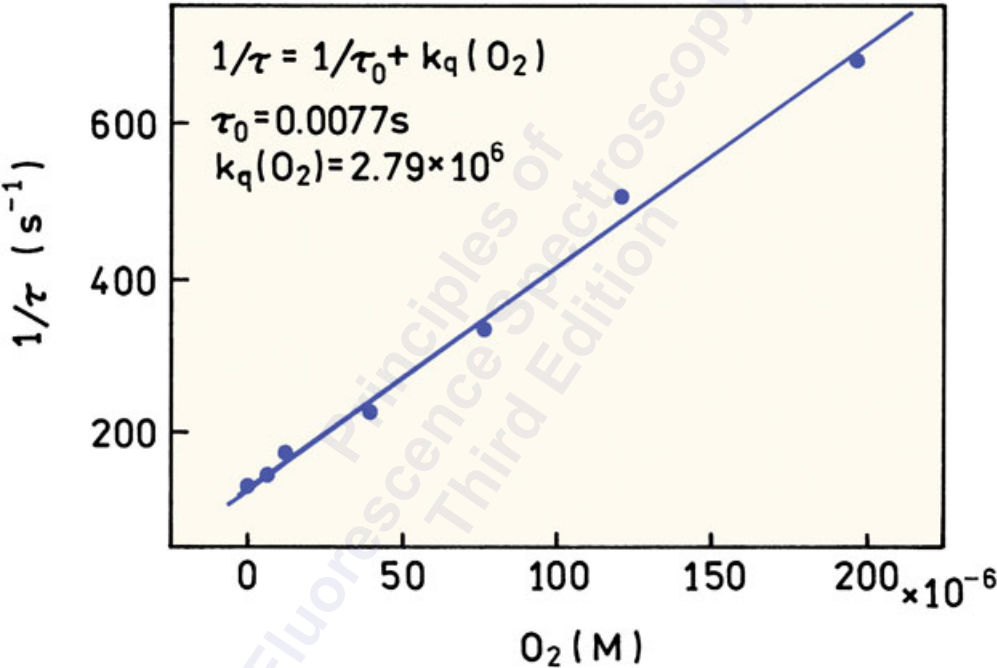


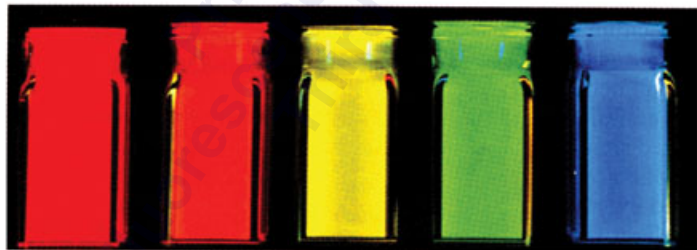
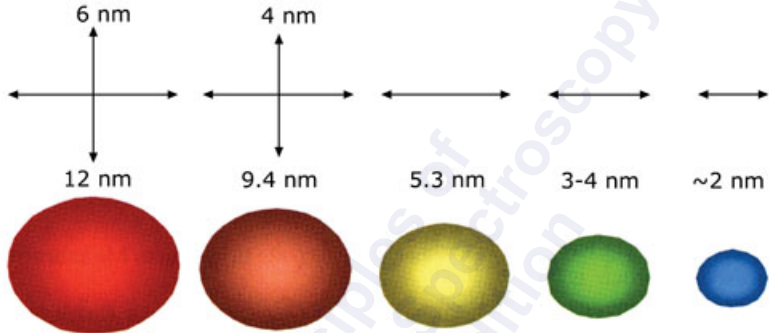


PHASE ( degrees )

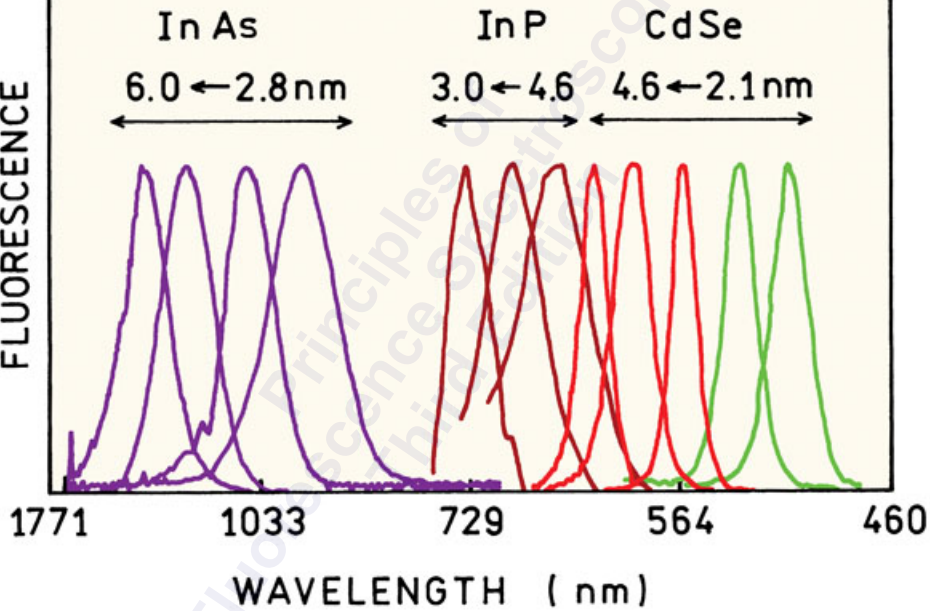
at 138.14 MHz

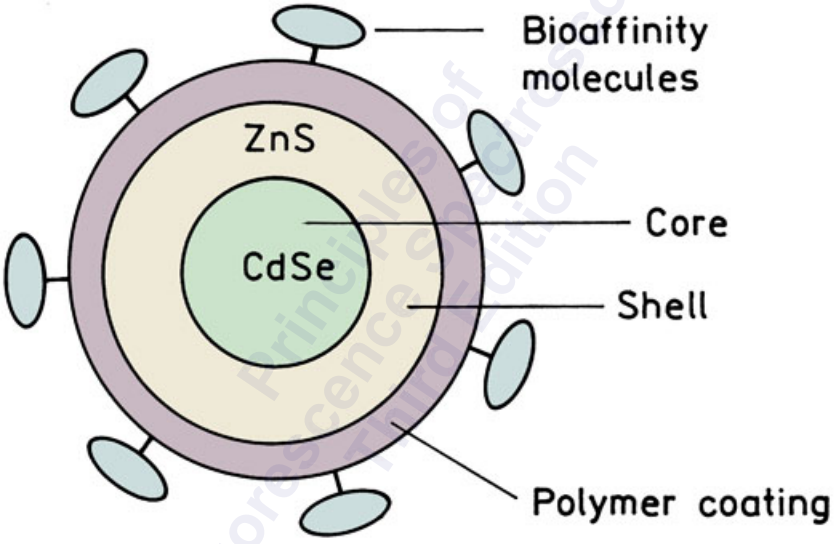




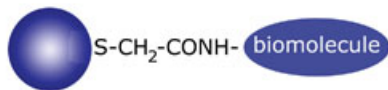


NORMALIZED  
FLUORESCENCE





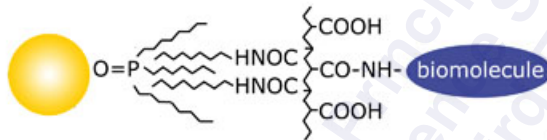
## Bifunctional linkage



## Electrostatic Attraction

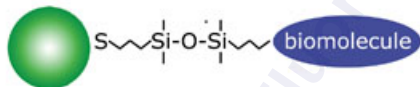


## Hydrophobic Attraction

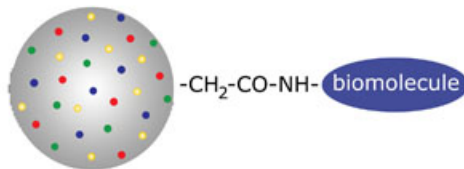


Peptides  
Proteins

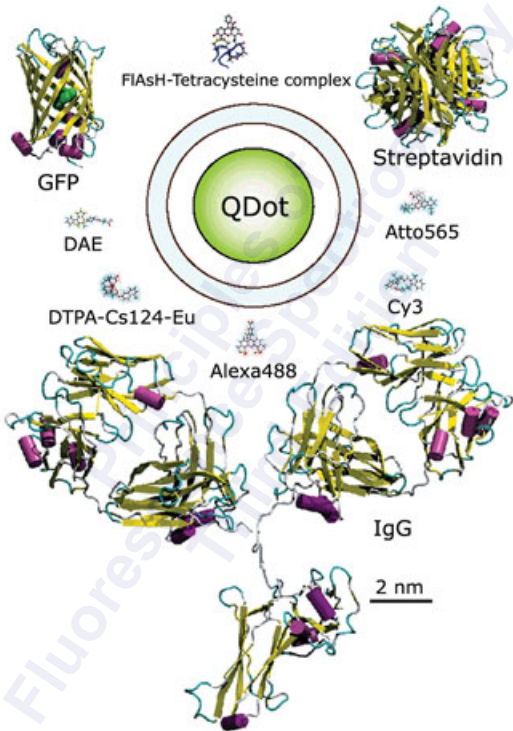
## Silanization



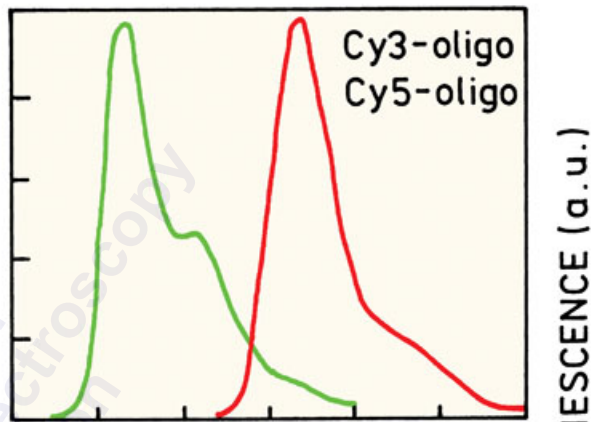
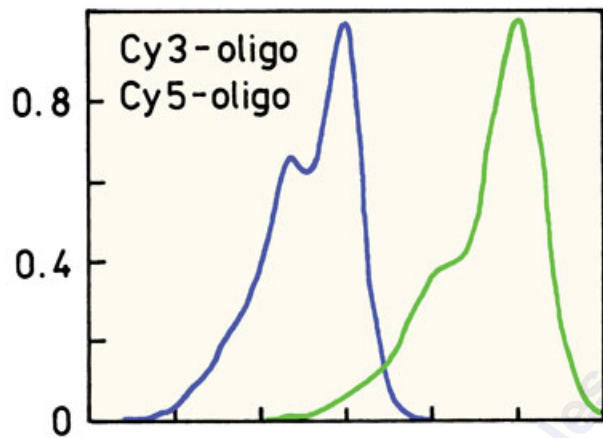
## Nanobeads



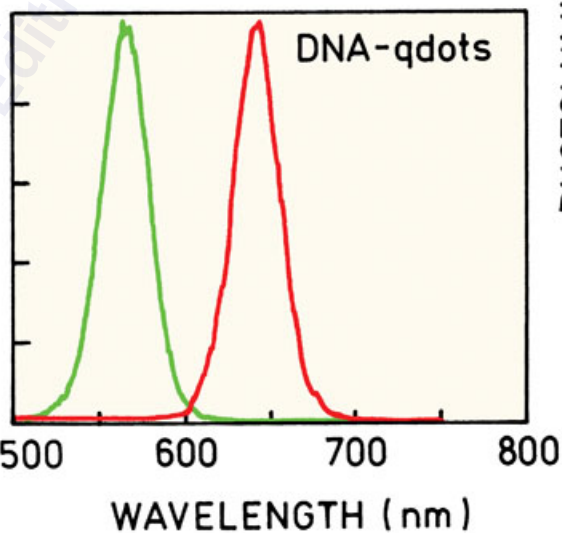
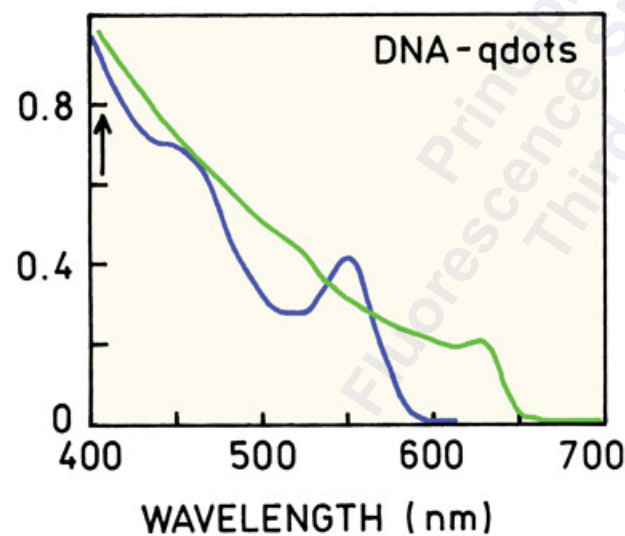


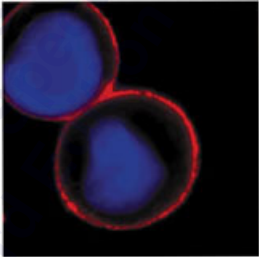
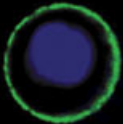


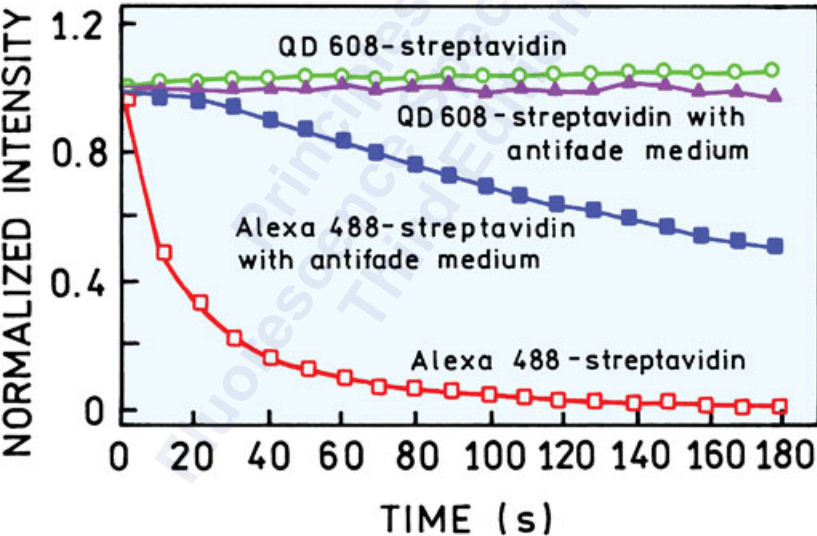
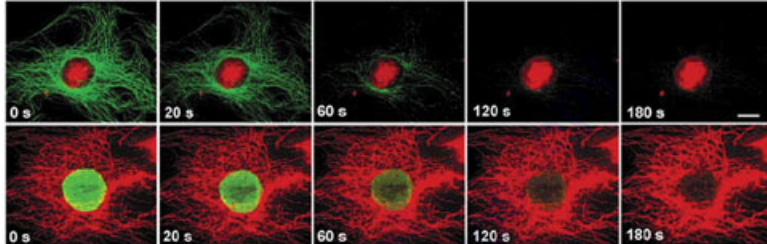
EXCITATION (a.u.)

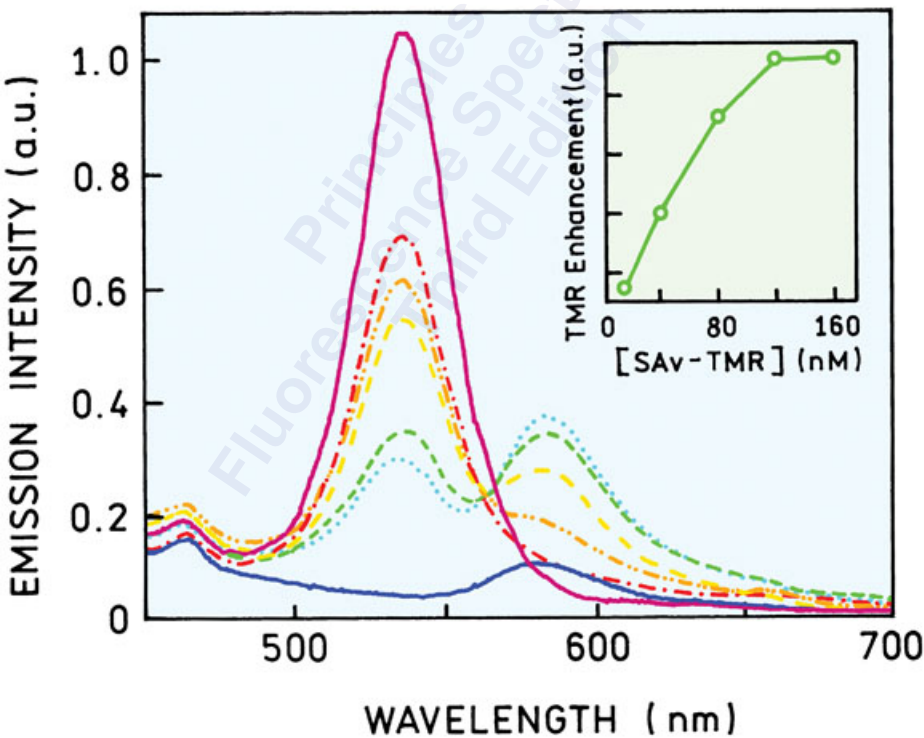
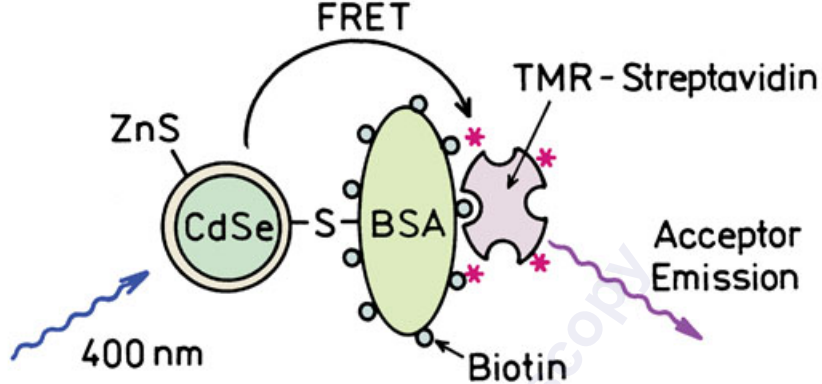


PHOTOLUMINESCENCE (a.u.)

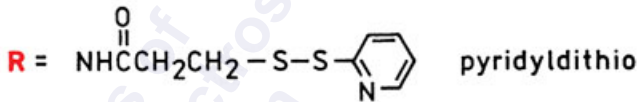
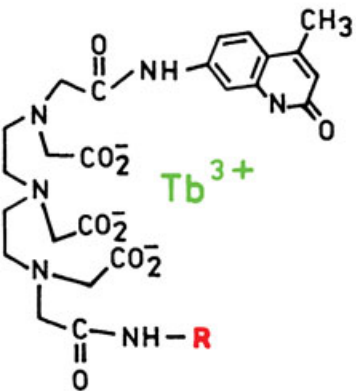




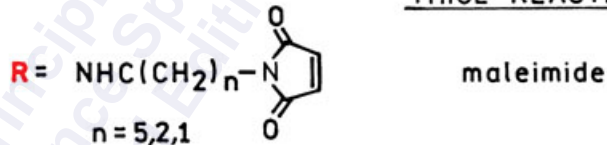




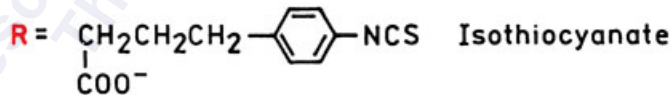
# DTPA - cs124 - R

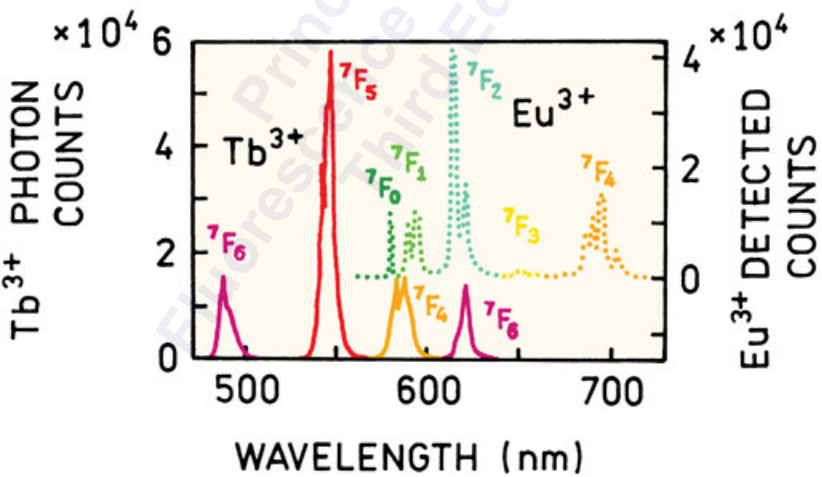
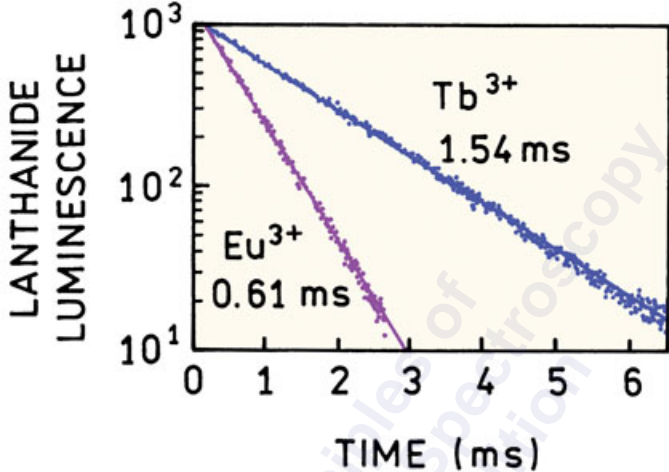


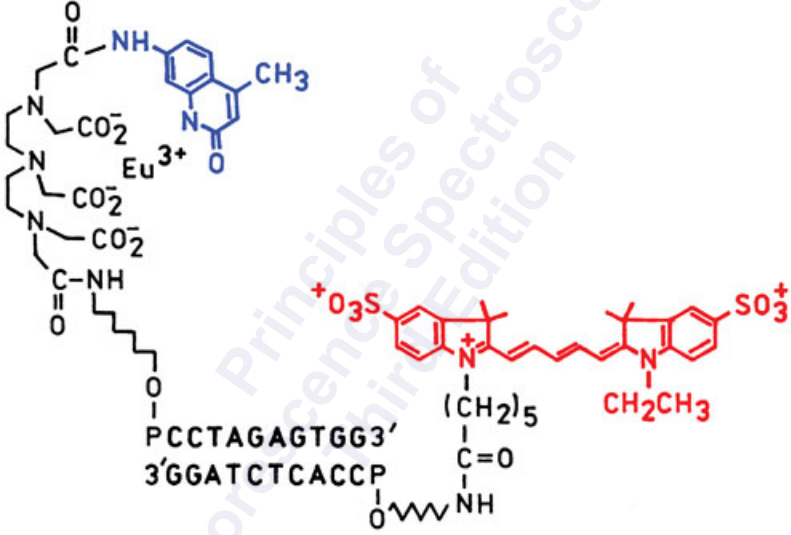
THIOL REACTIVE



AMINE REACTIVE

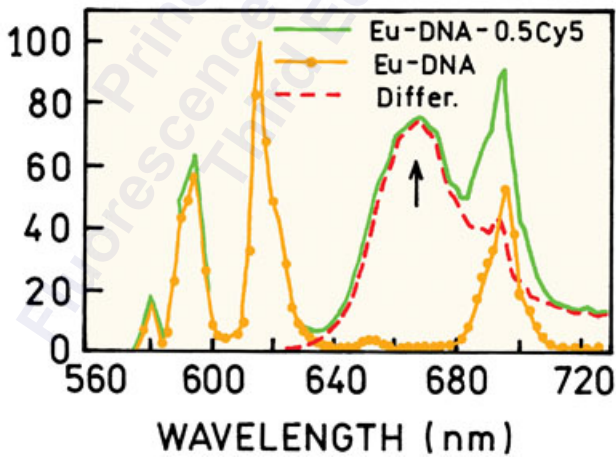
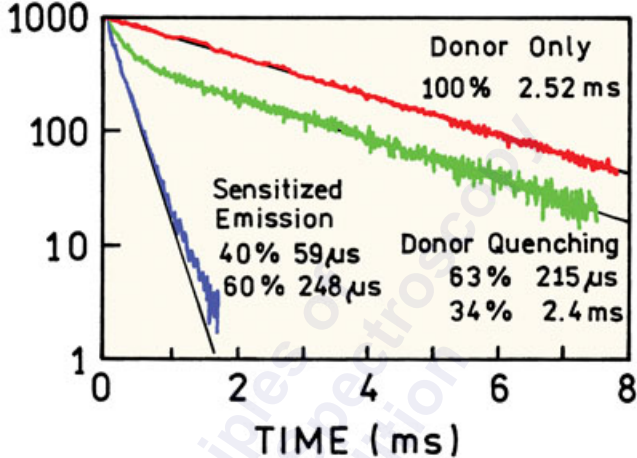


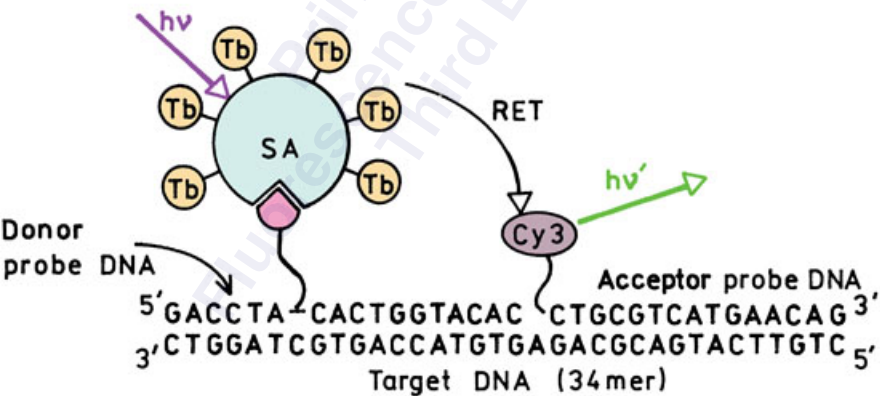
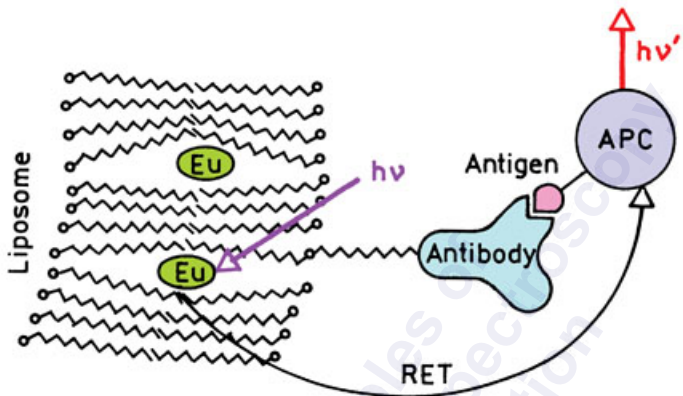


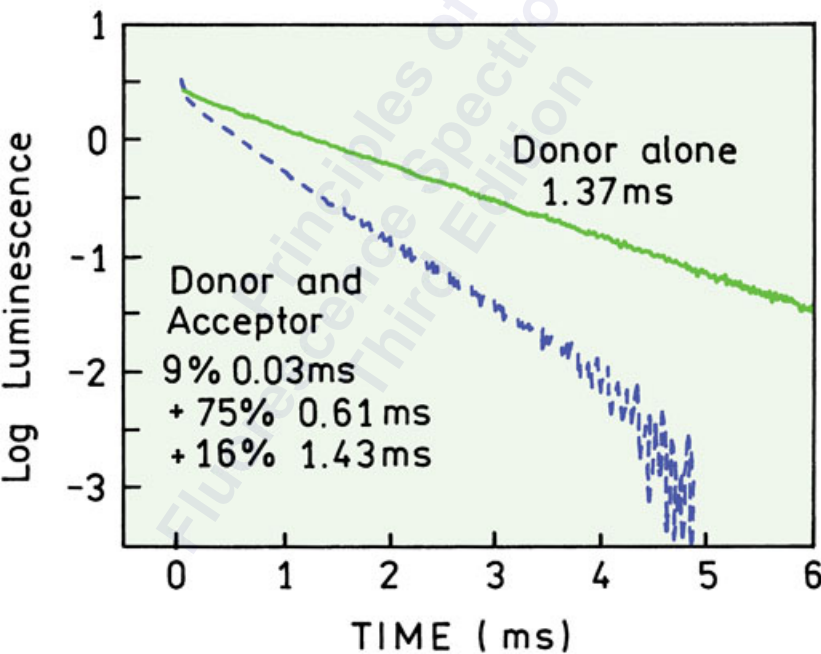
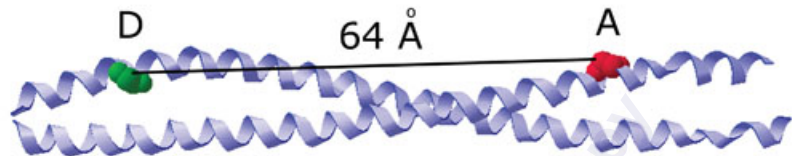


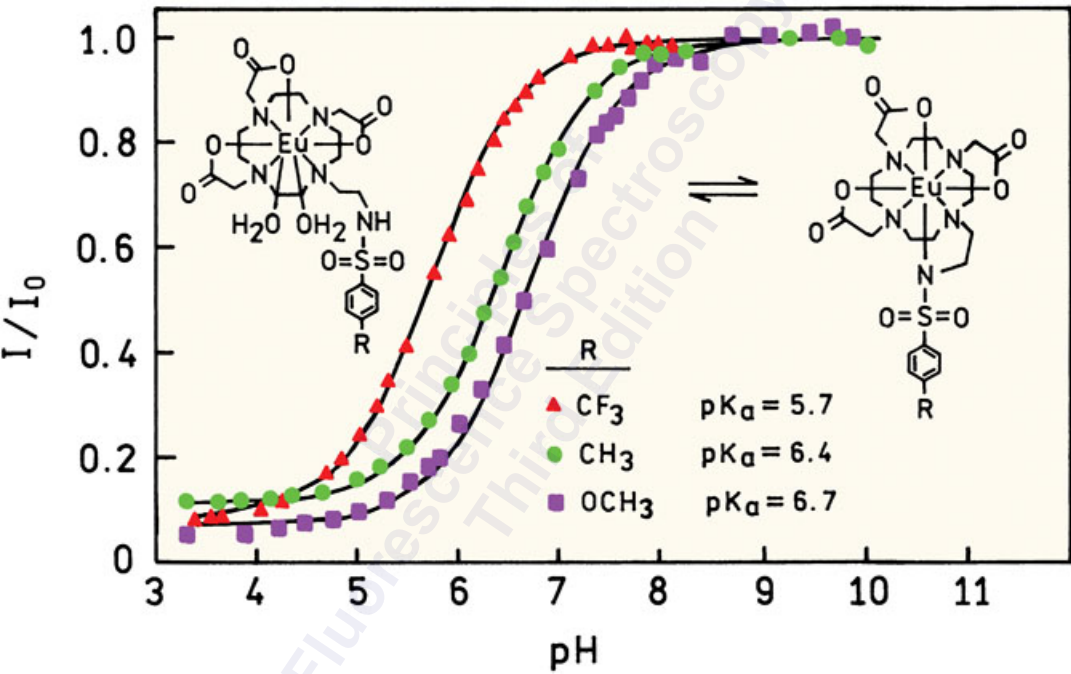


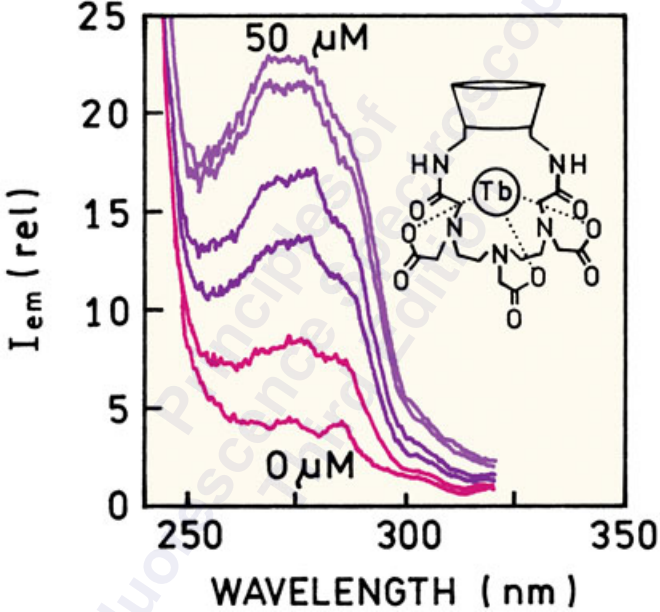
NORMALIZED EMISSION

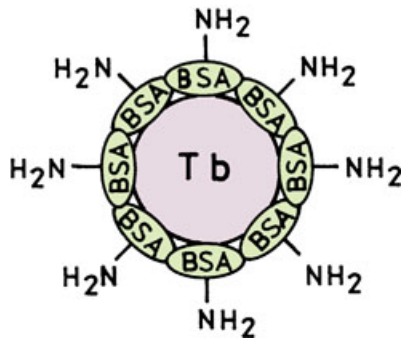
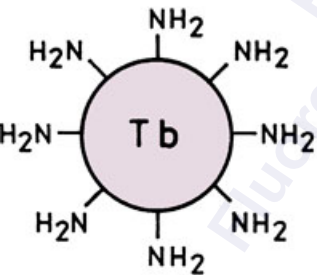
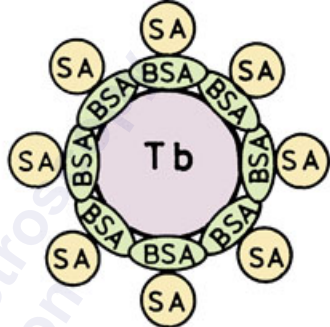
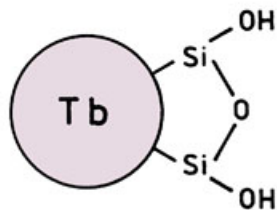




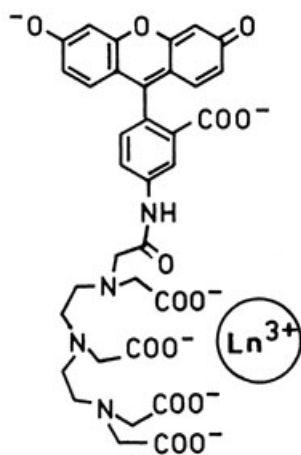




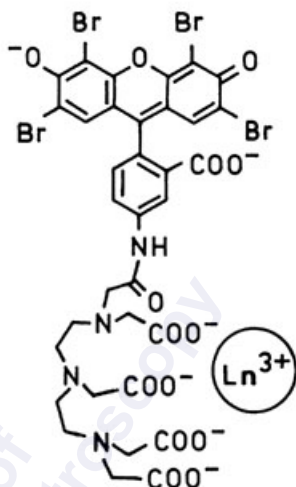




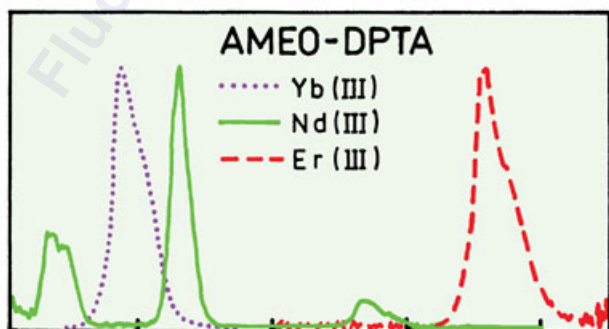
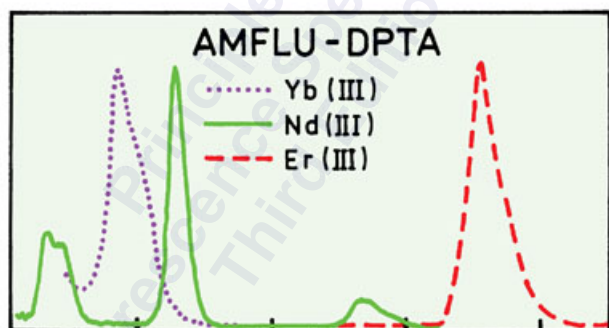
AMFLU-DTPA



AMEO-DTPA

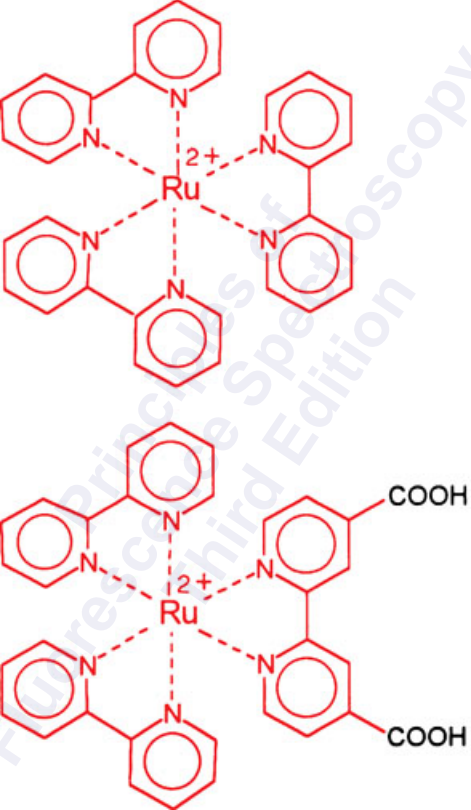


FLUORESCENCE INTENSITY

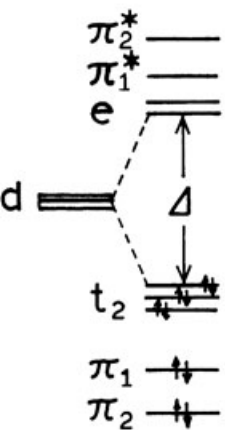


1000 1200 1400 1600

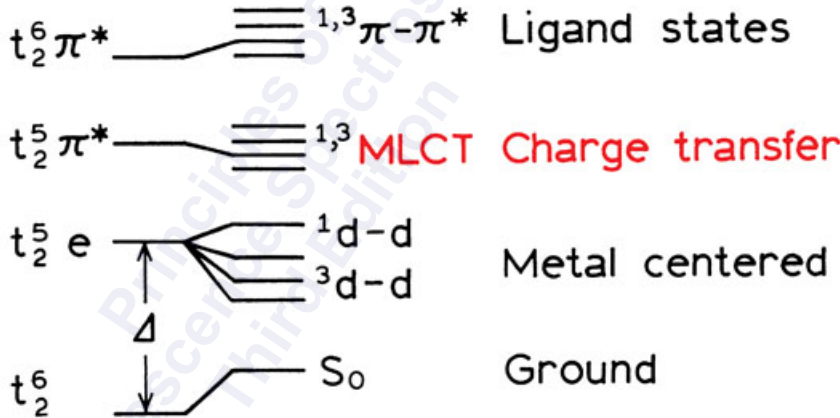
WAVELENGTH (nm)



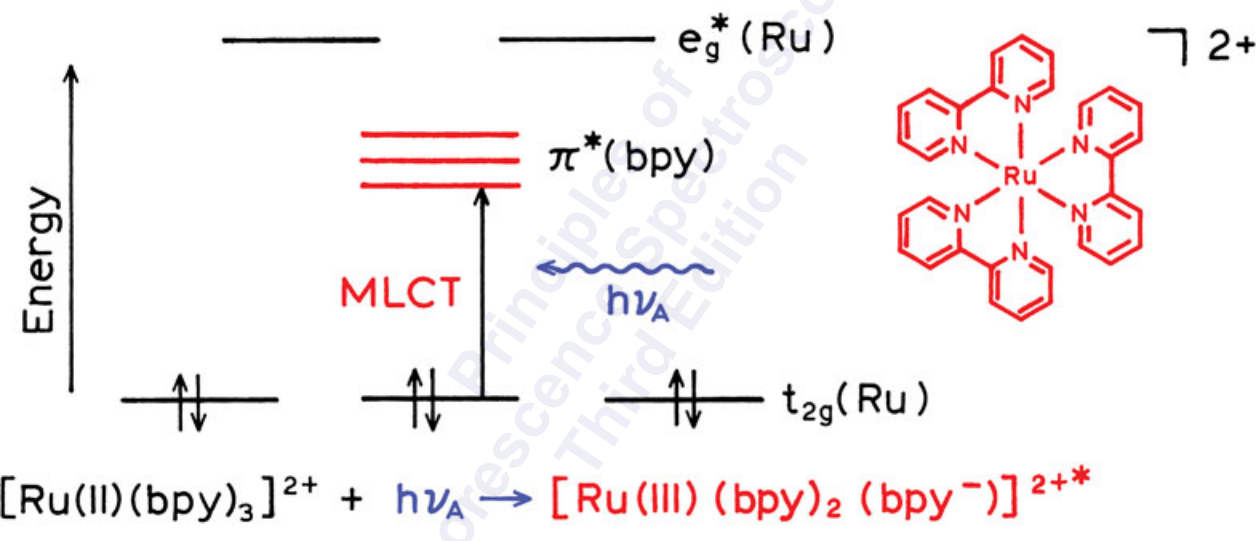


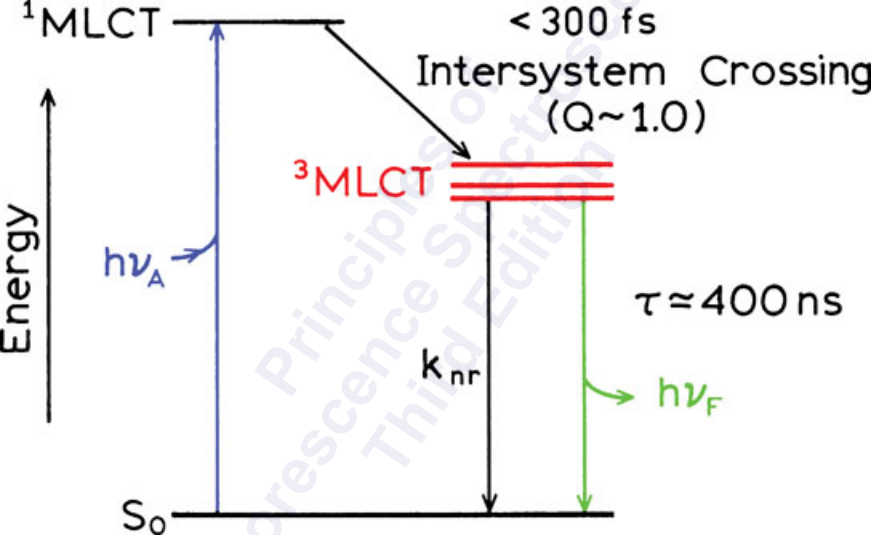


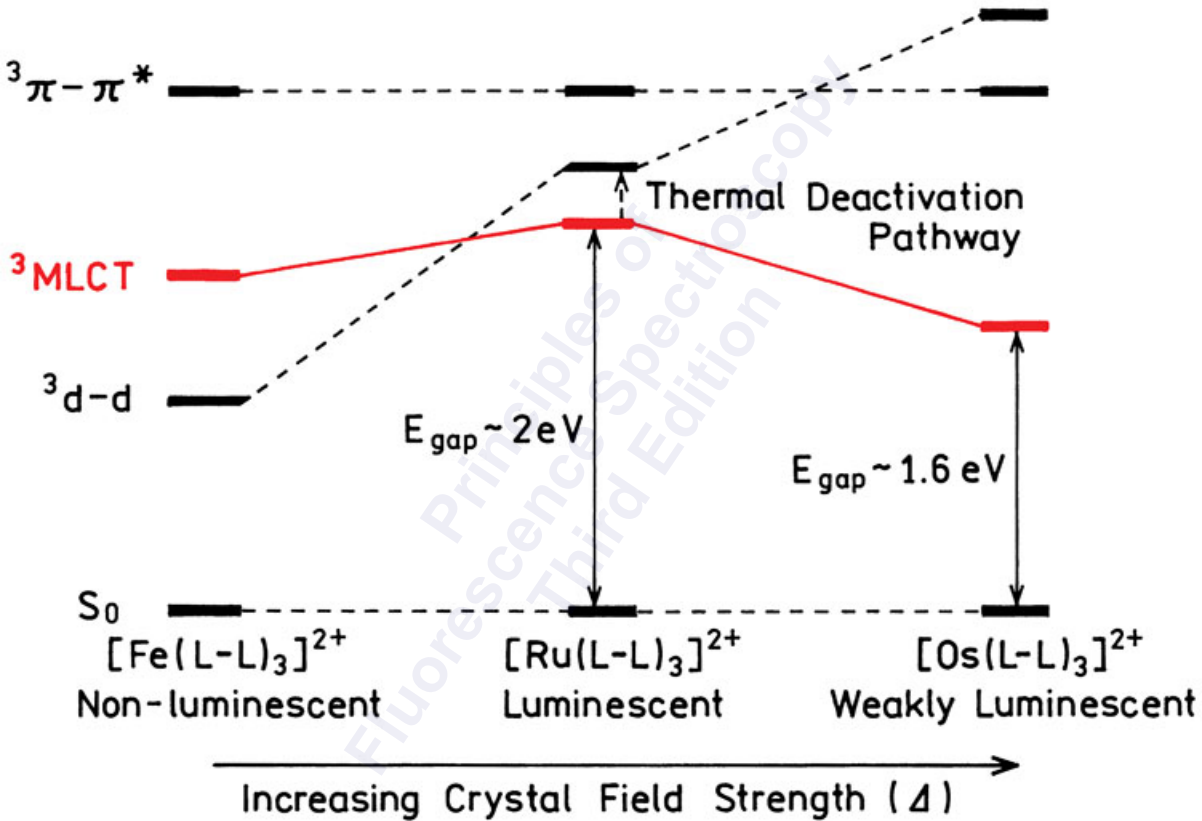
ORBITALS

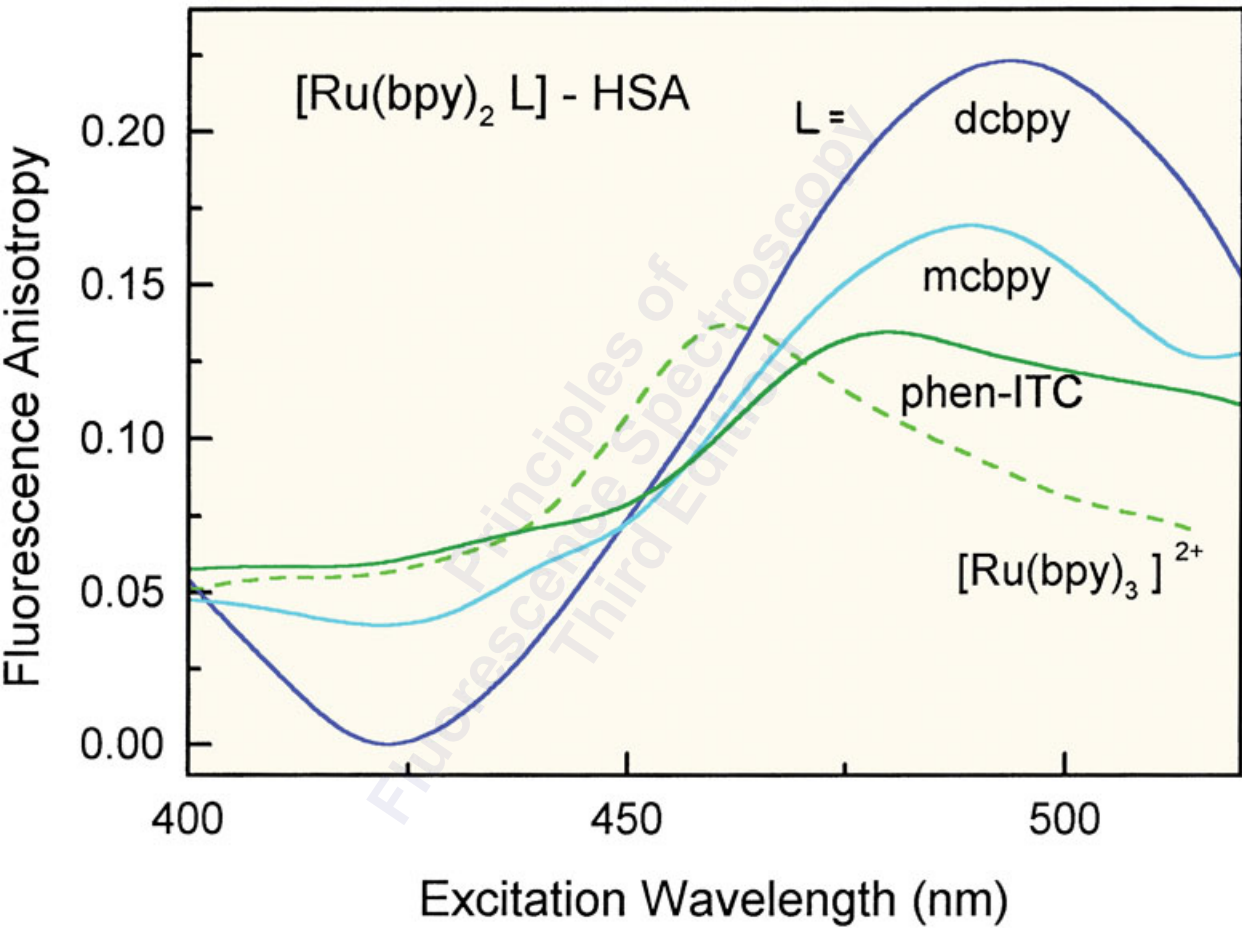


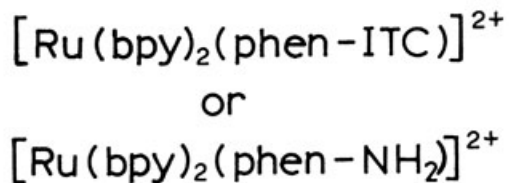
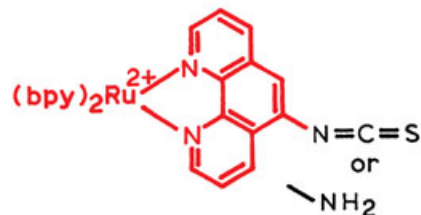
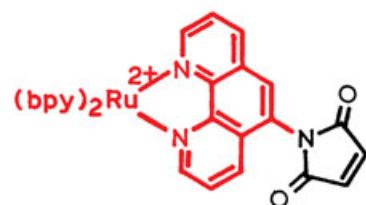
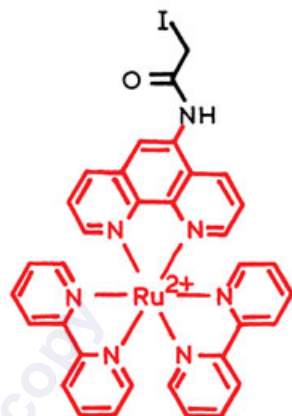
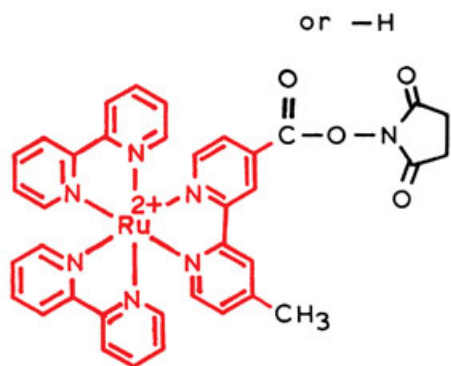
STATES

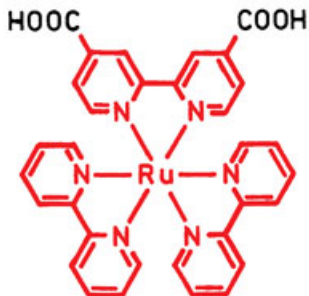
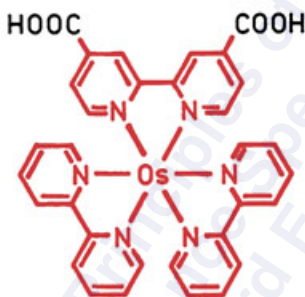
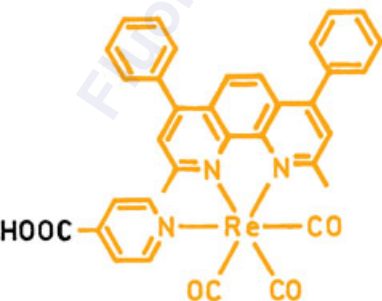
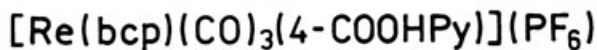


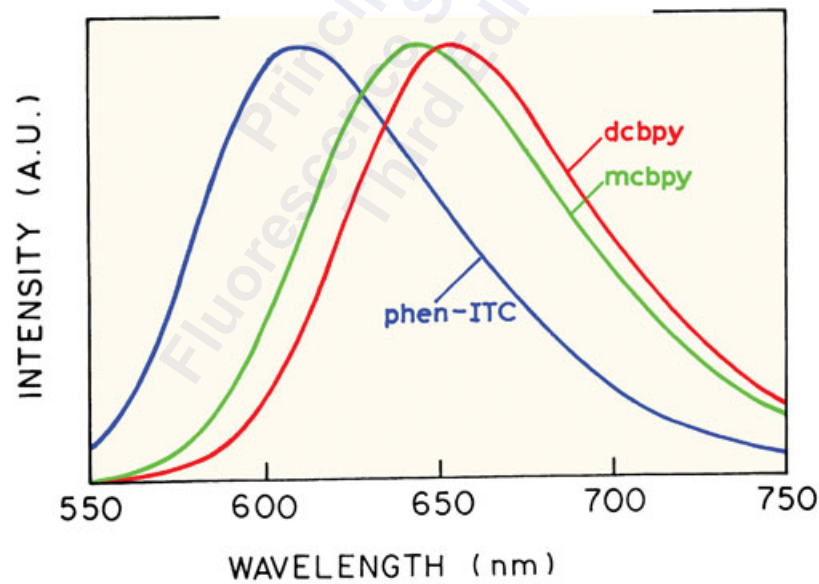
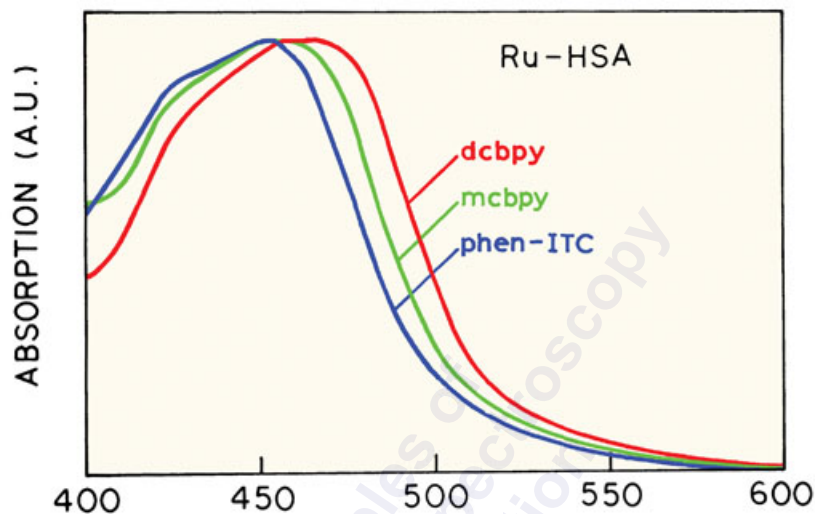




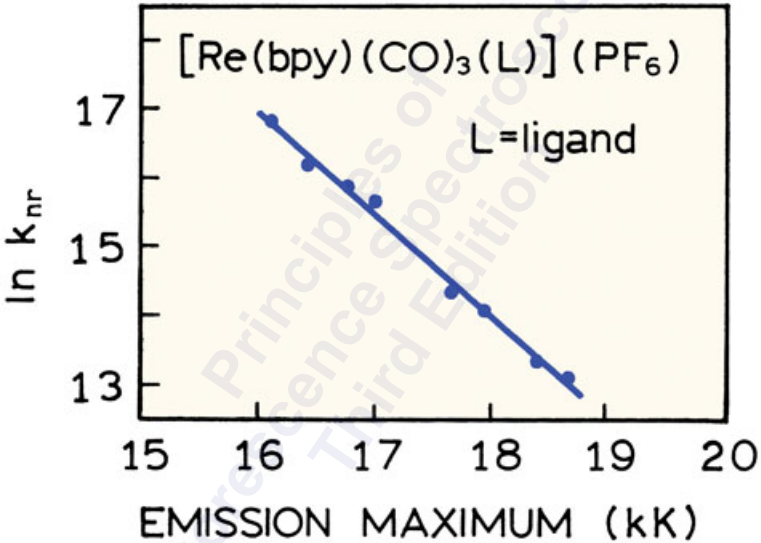


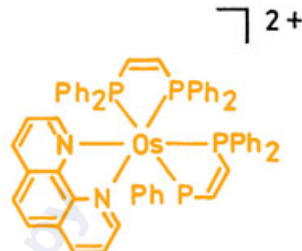
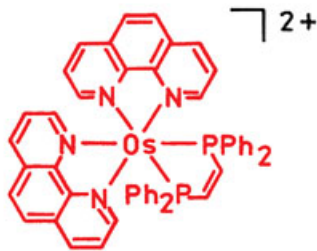
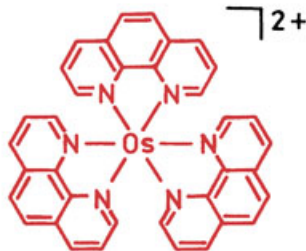
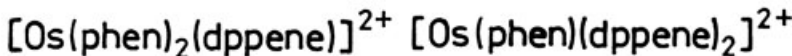
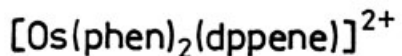



 $\lceil 2+$ 
 $\tau = 336 \text{ ns}$   
 $Q = 0.0035$ 

 $\lceil 2+$ 
 $\tau = 18 \text{ ns}$   
 $Q < 0.005$ 

 $\lceil +$ 
 $\tau = 2700 \text{ ns}$   
 $Q = 0.20$ 


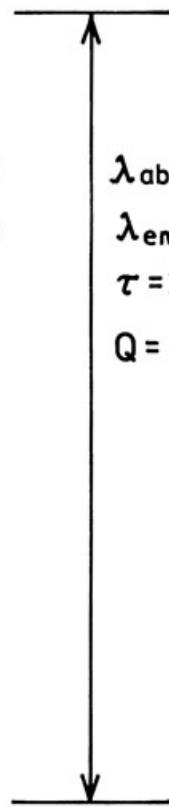
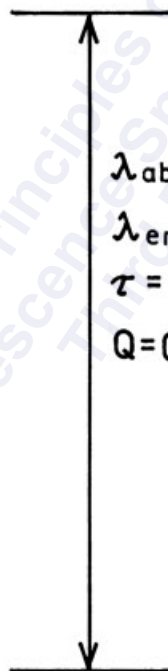
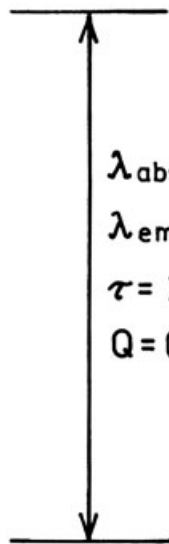


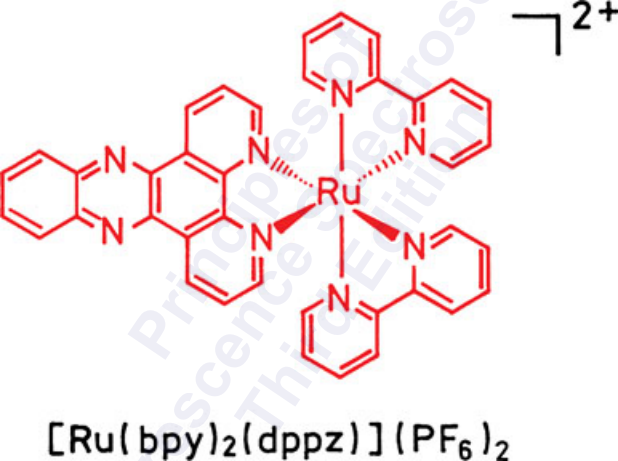


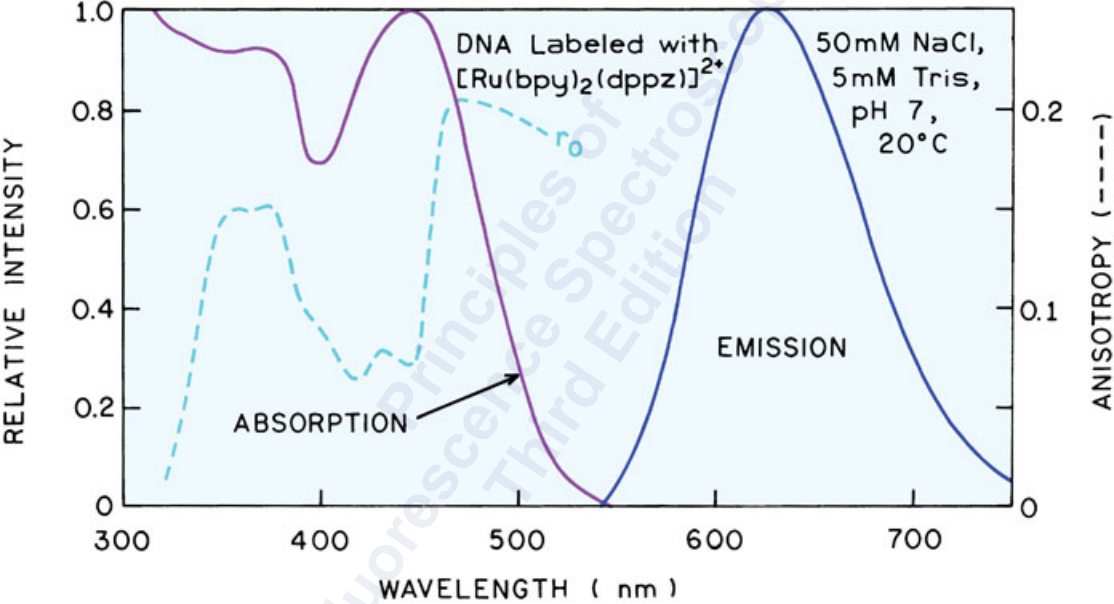


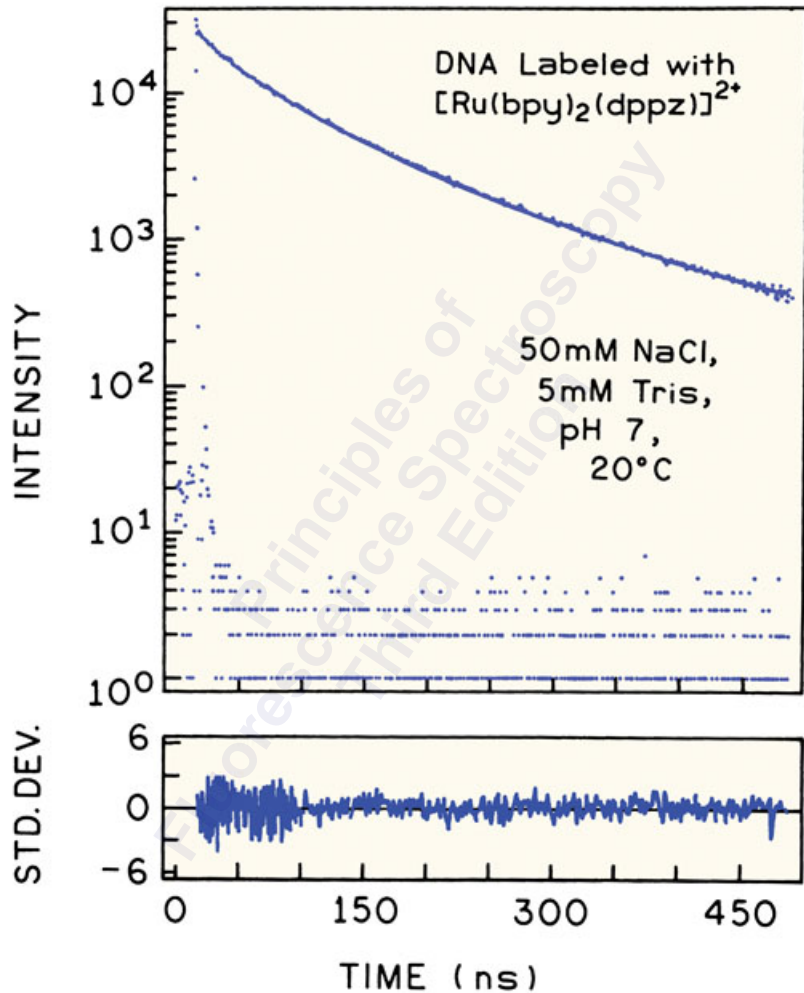


Relative Energy Gap Magnitude

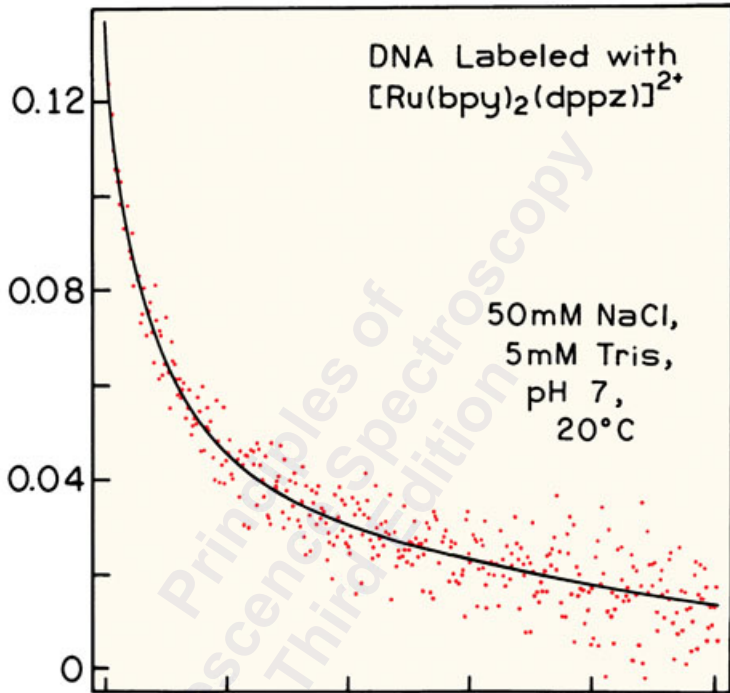




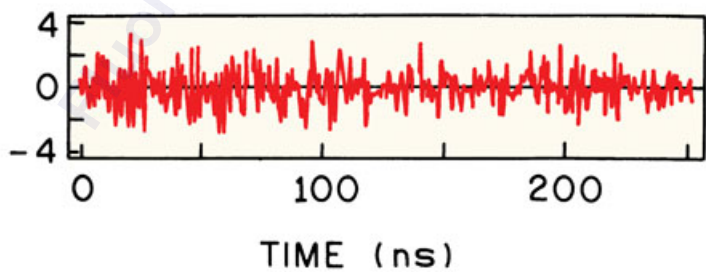


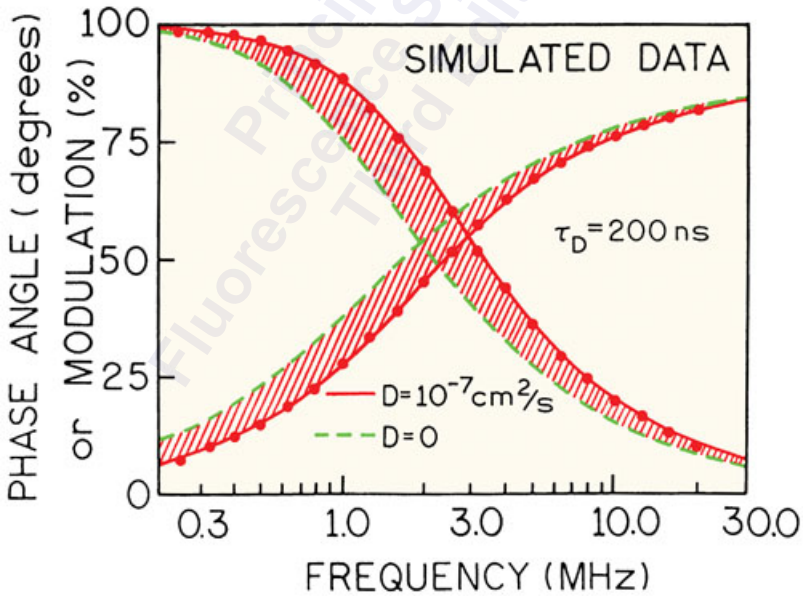
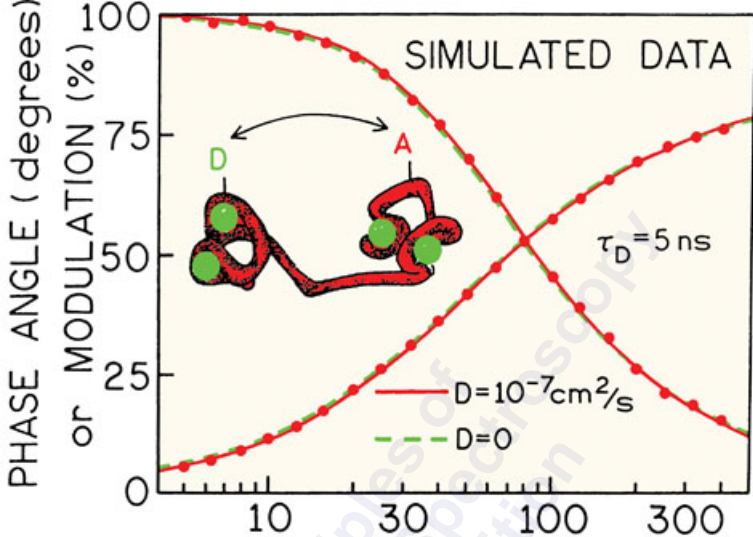


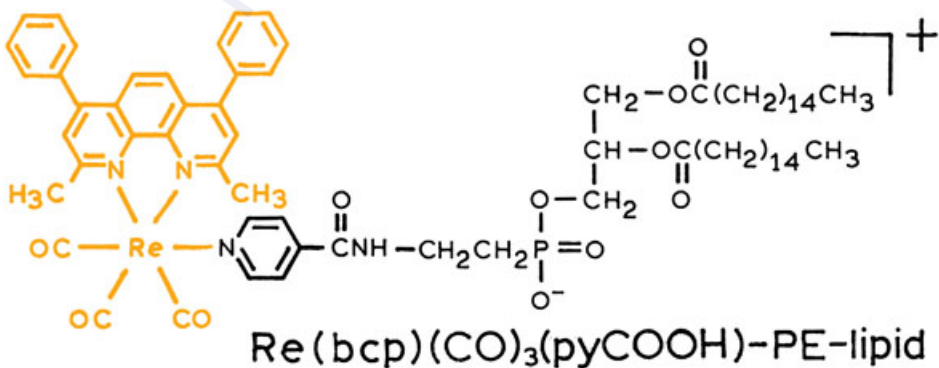
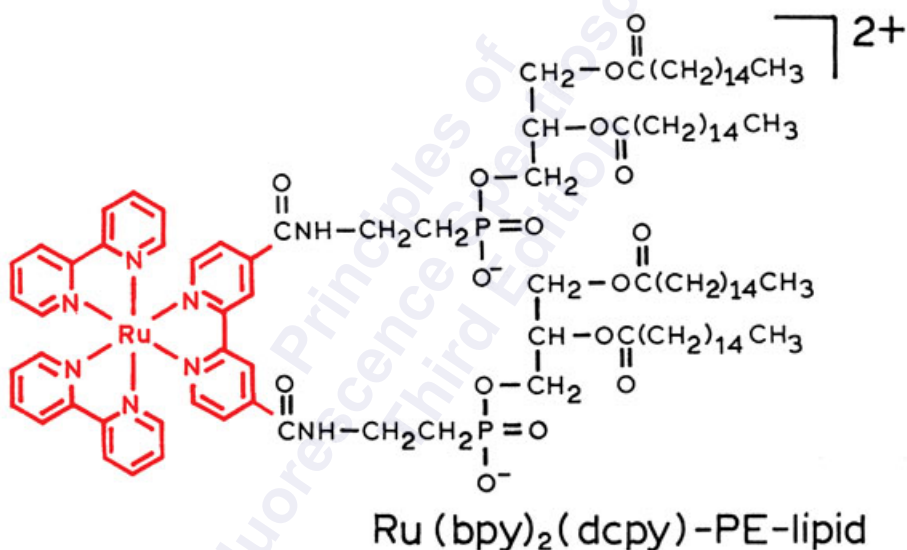
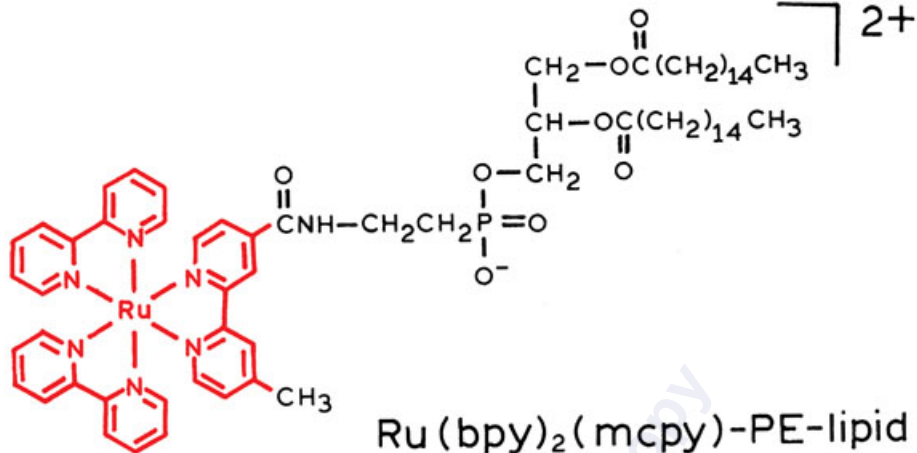
ANISOTROPY



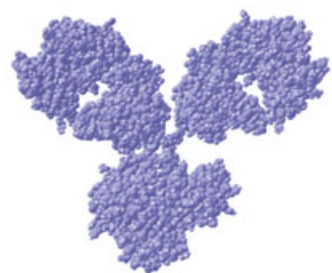
STD. DEV.



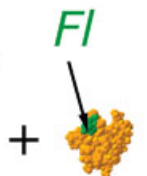






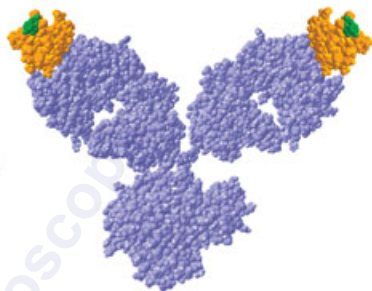
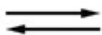


IgG

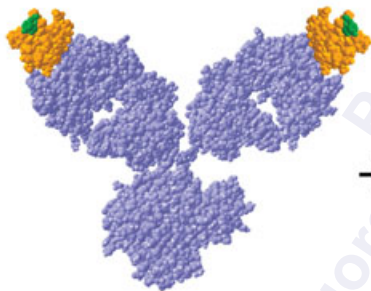


FI-A

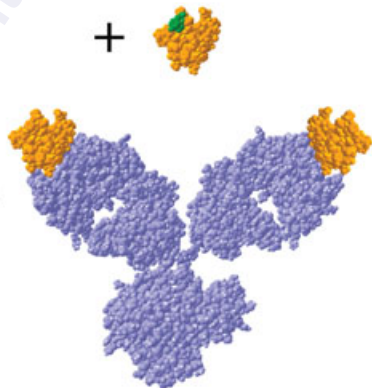
$r \approx 0$



$r \approx r_0$

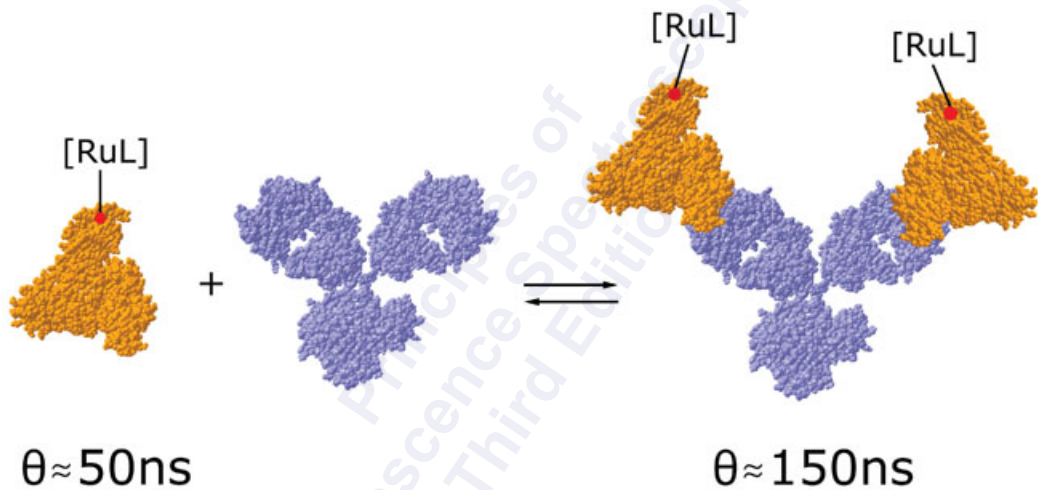


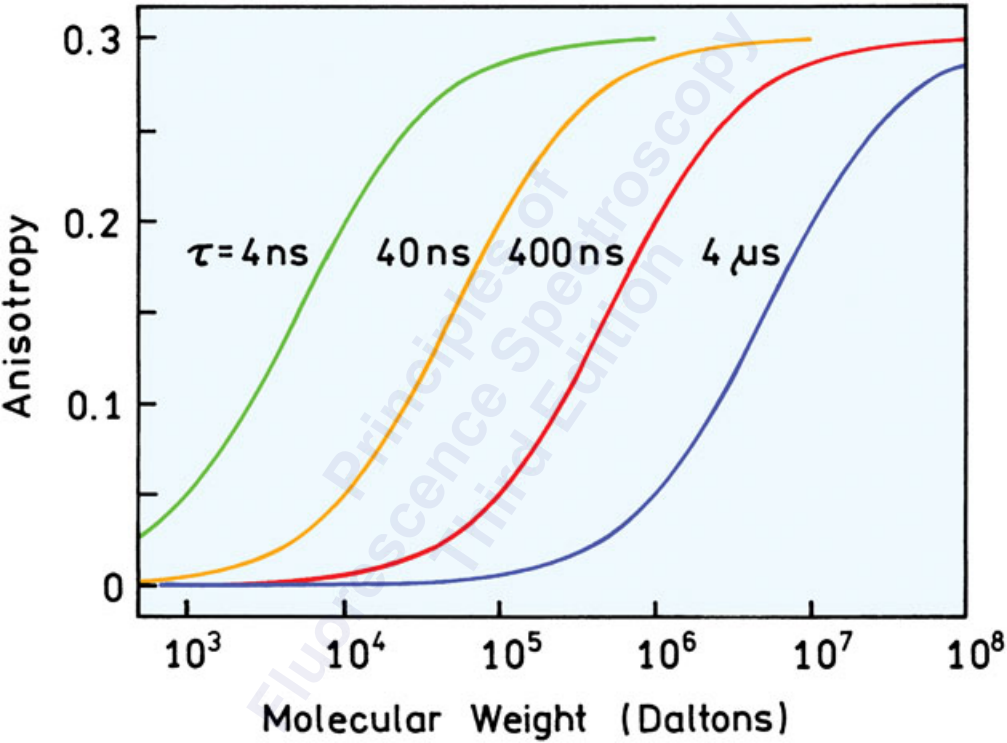
$r \approx r_0$



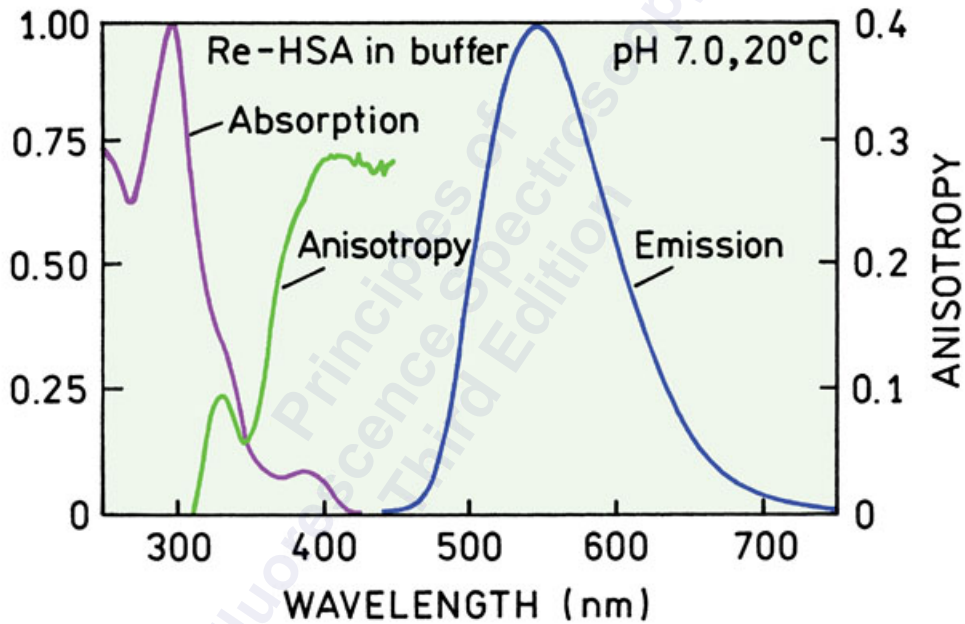
$r \approx 0$

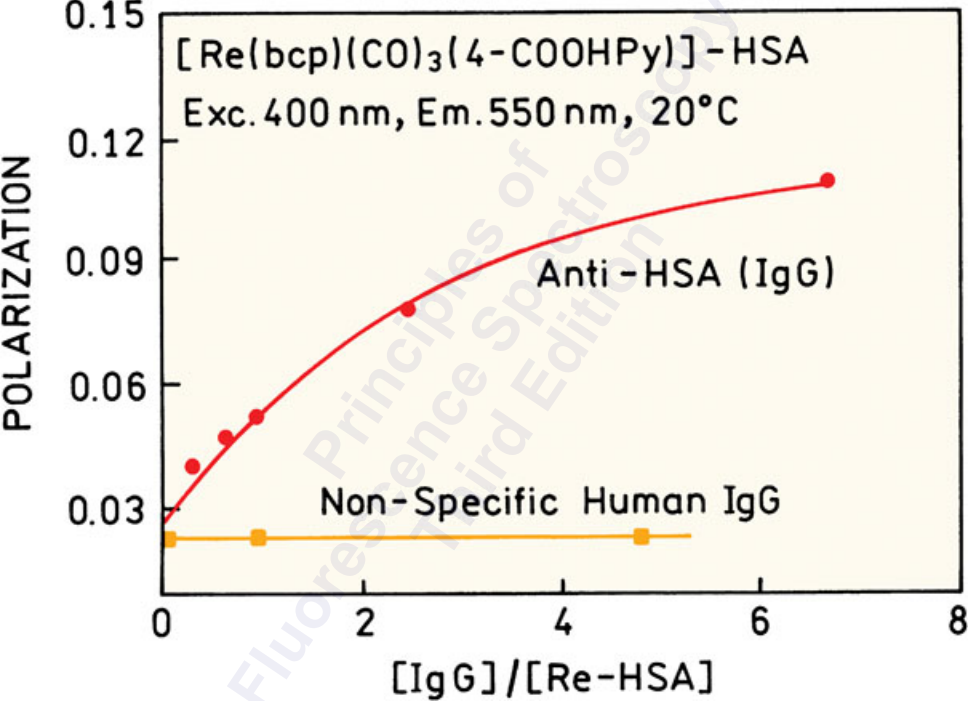
Fluorescence Spectroscopy  
Third Edition

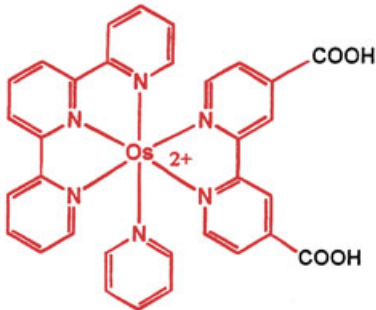




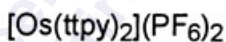
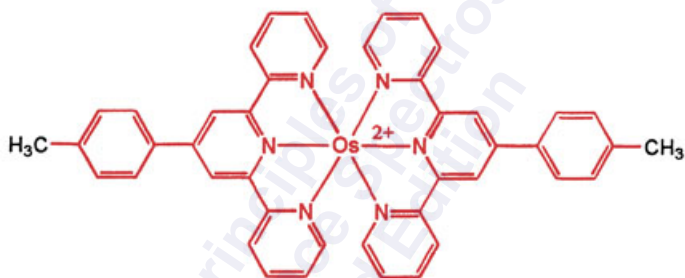
RELATIVE INTENSITY



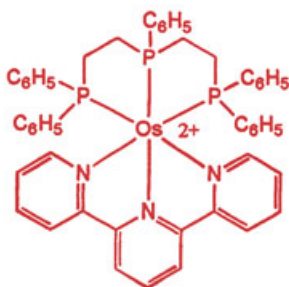




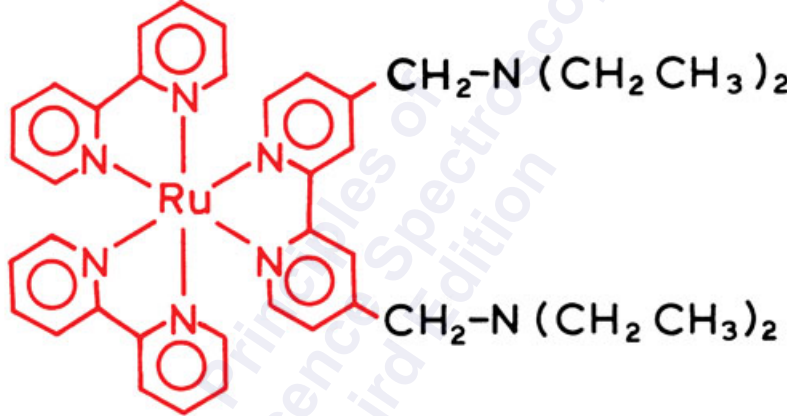
$$\lambda_{\text{abs}} = 550 \text{ nm}, \lambda_{\text{em}} = 640 \text{ nm}, \tau = 130 \text{ ns}$$



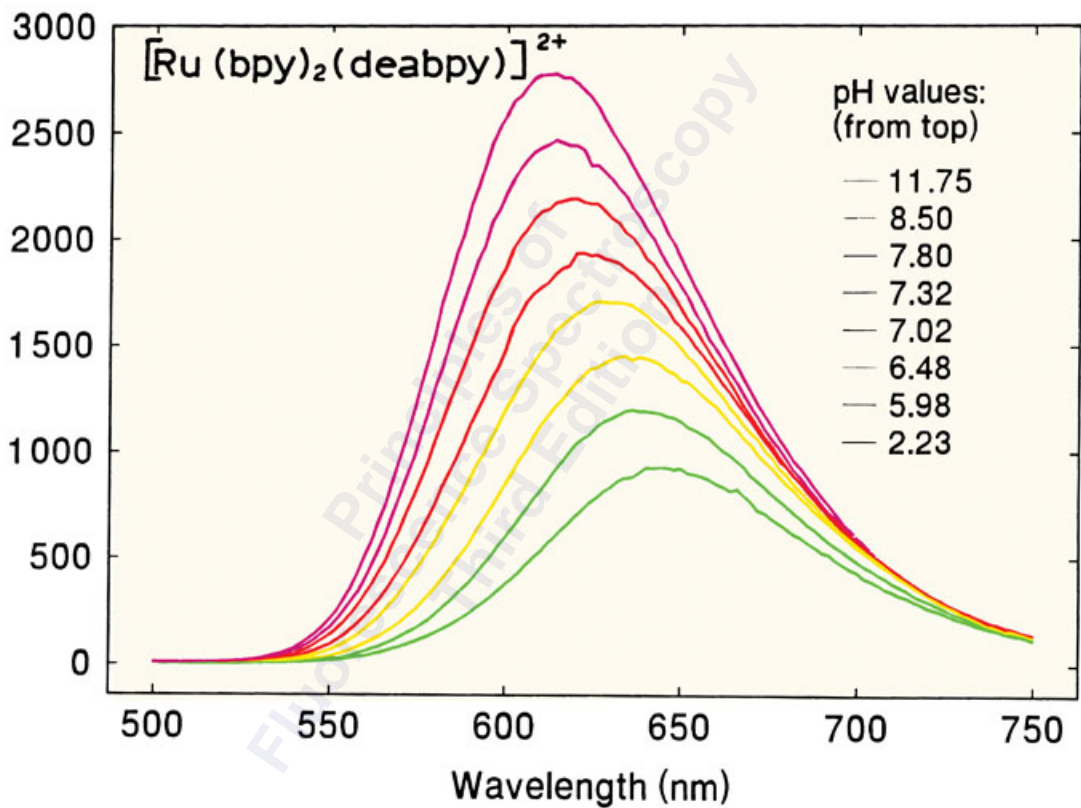
$$\lambda_{\text{abs}} = 600 \text{ nm}, \lambda_{\text{em}} = 735 \text{ nm}, \tau = 220 \text{ ns}$$



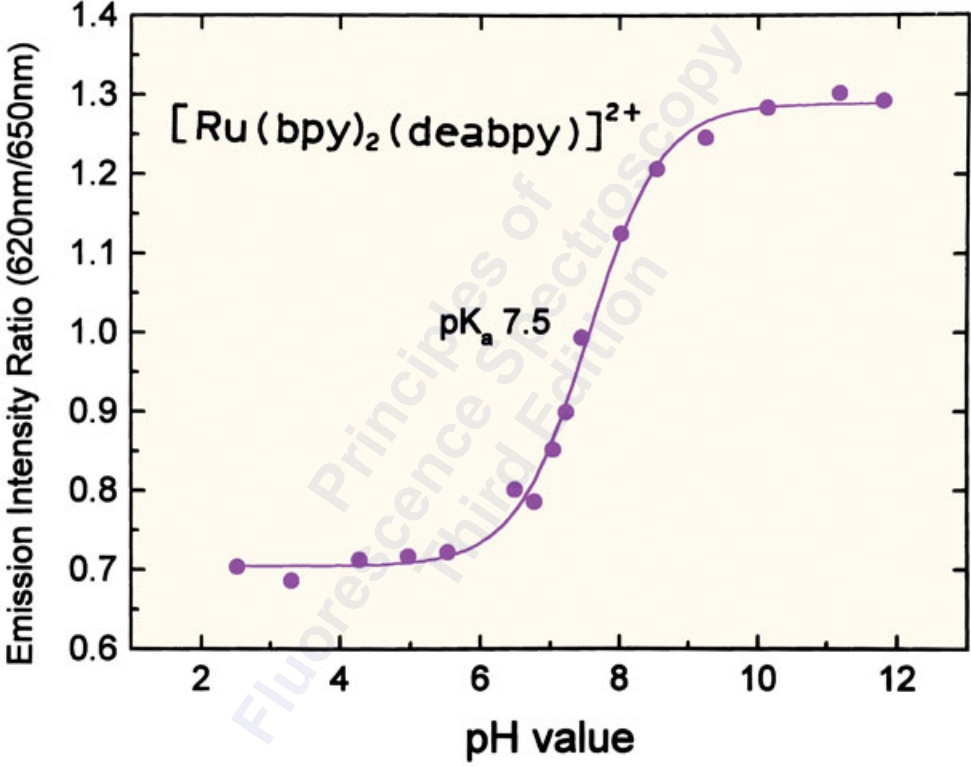
$$\lambda_{\text{abs}} = 470 \text{ nm}, \lambda_{\text{em}} = 710 \text{ nm}, \tau = 230 \text{ ns}$$

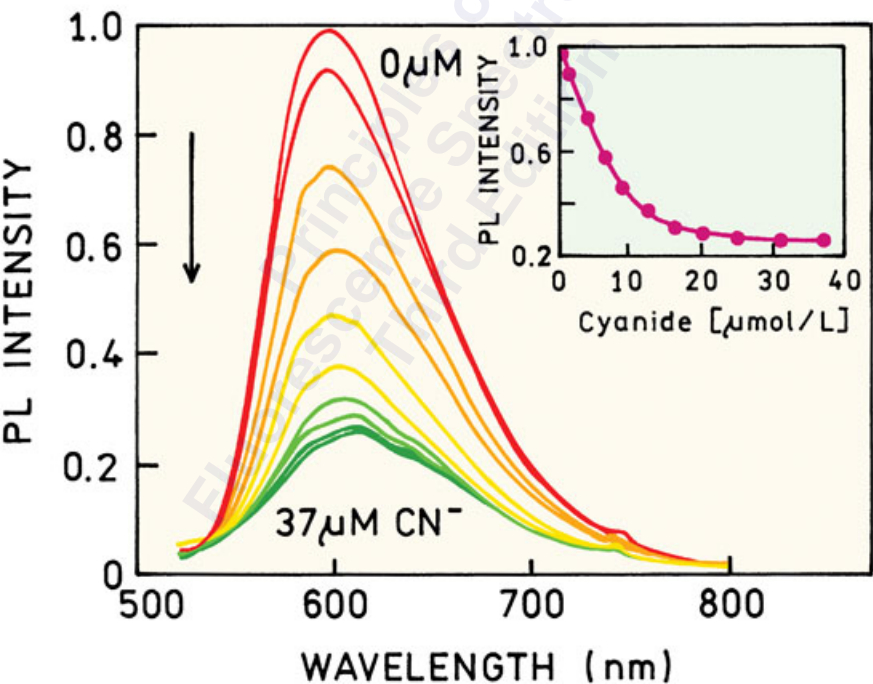
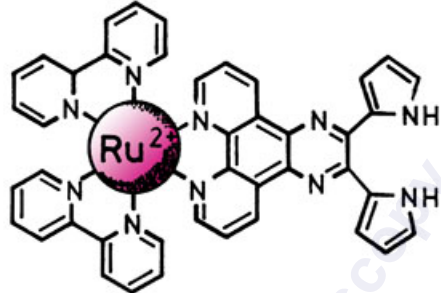


Fluorescence intensity



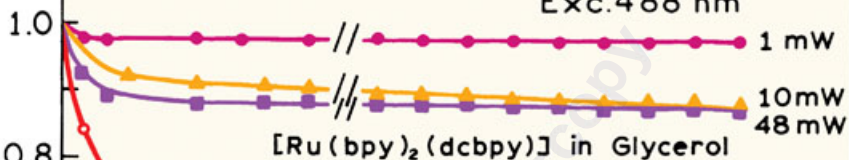




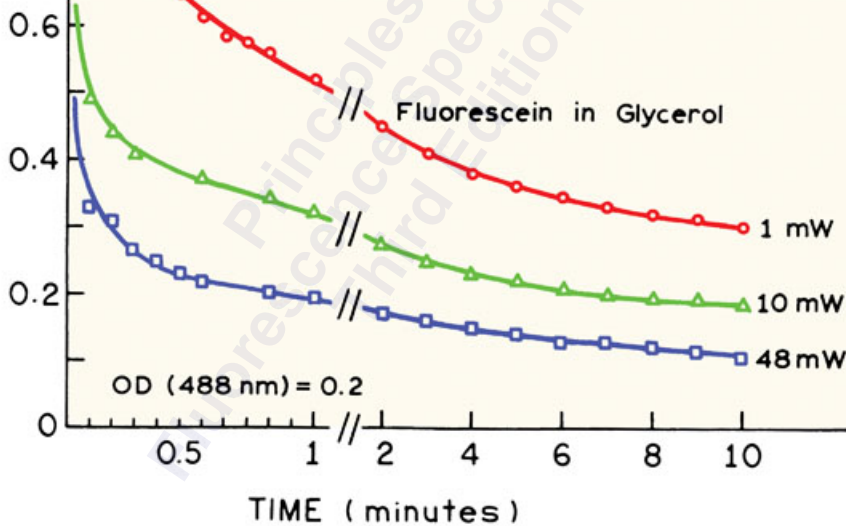


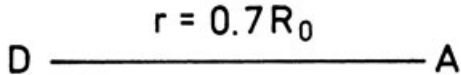
FRACTION OF INITIAL INTENSITY

Exc. 488 nm



Fluorescein in Glycerol





$$\tau_D = 1000 \text{ ns}$$

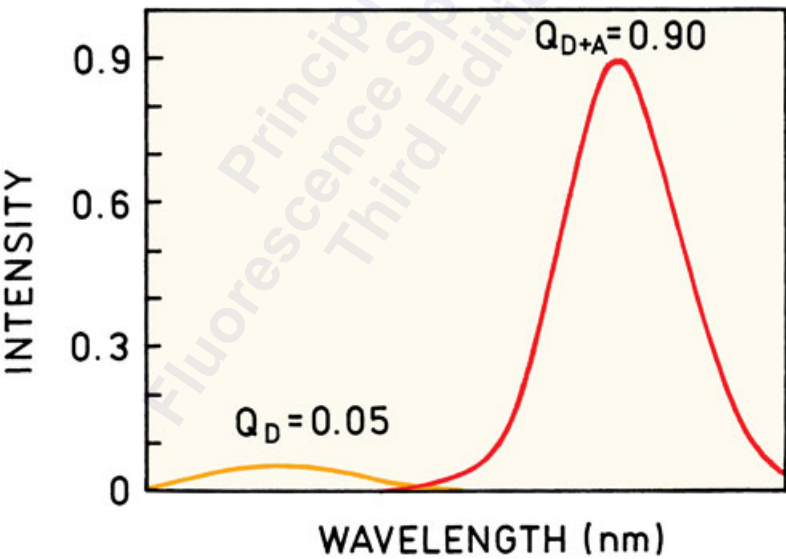
$$\tau_A = 1 \text{ ns}$$

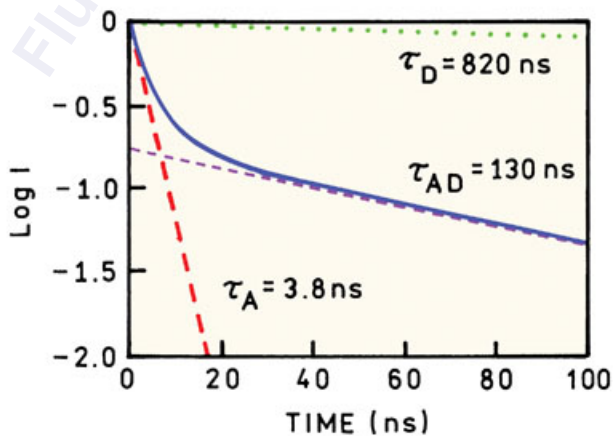
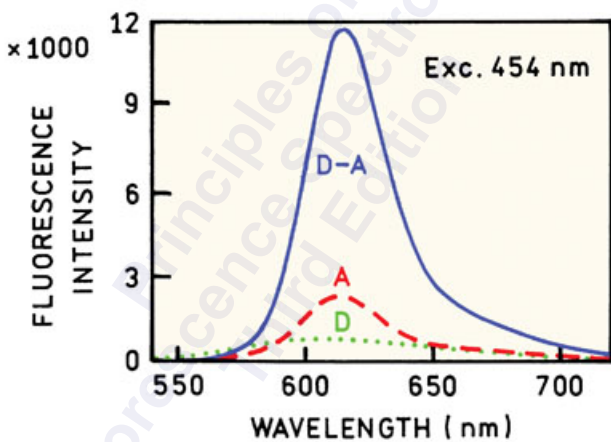
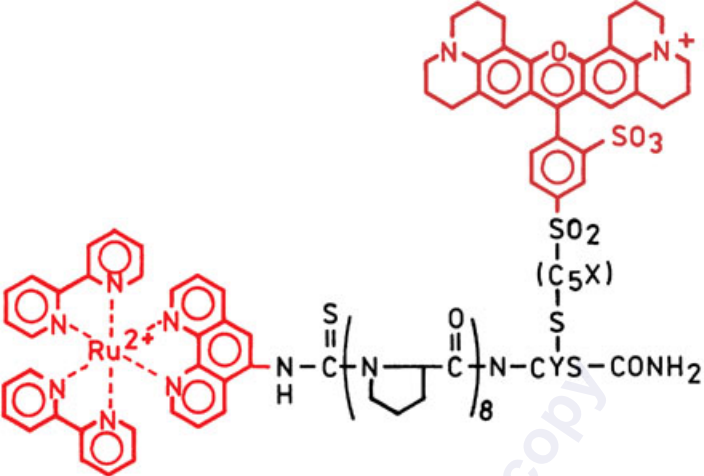
$$\tau_{DA} = 100 \text{ ns}$$

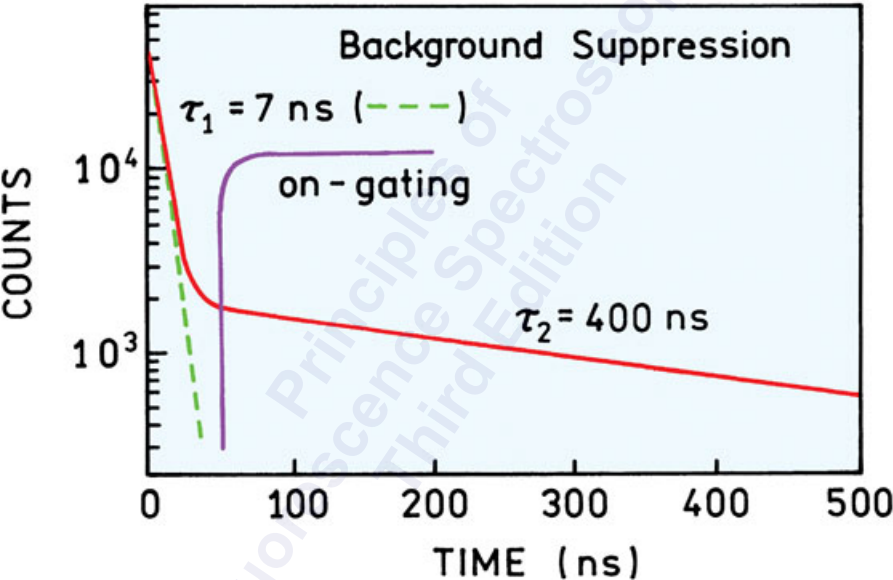
$$\tau_{AD} = 100 \text{ ns}$$

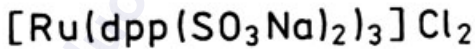
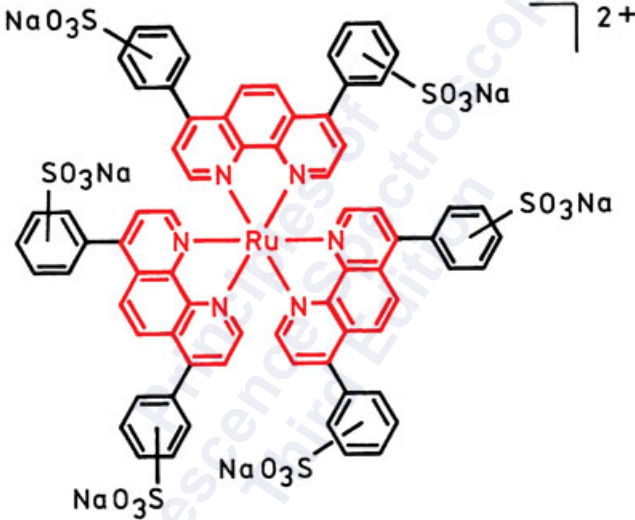
$$Q_D = 0.05$$

$$Q_A = 1.0$$

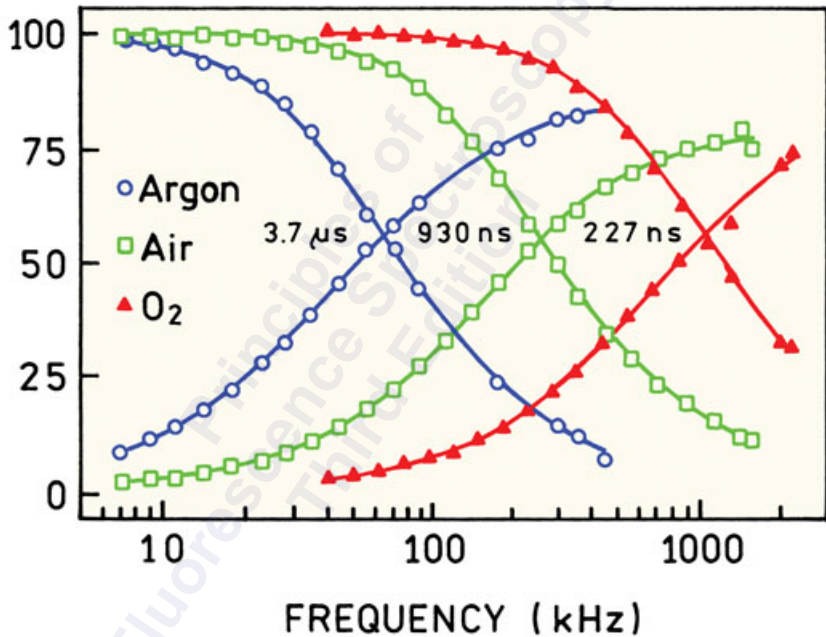




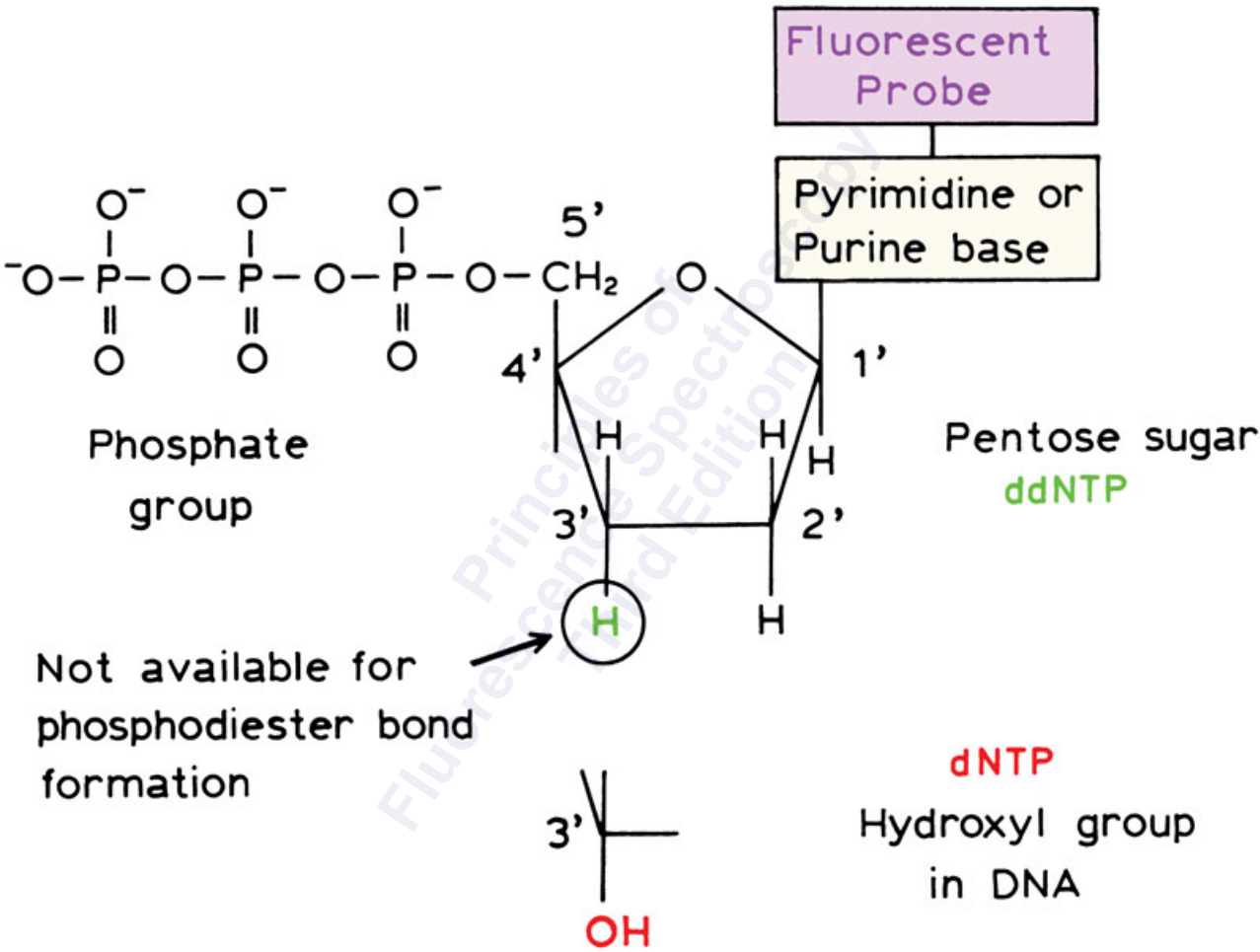


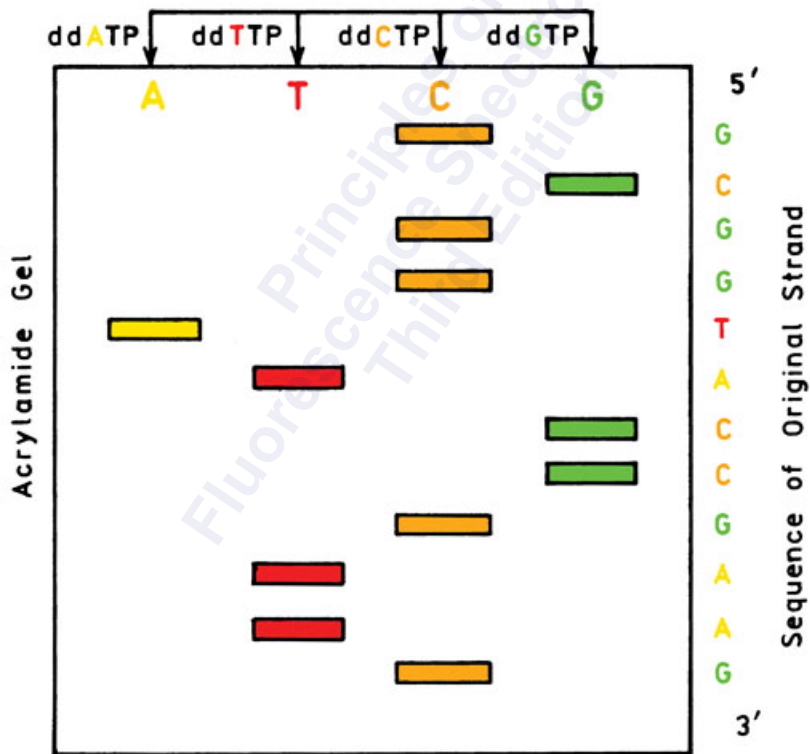
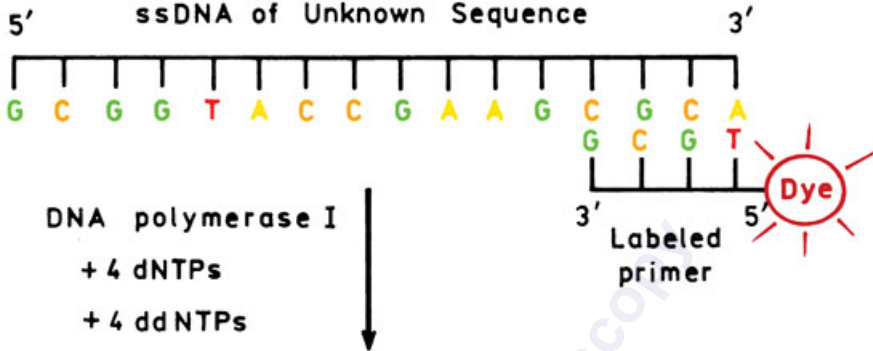


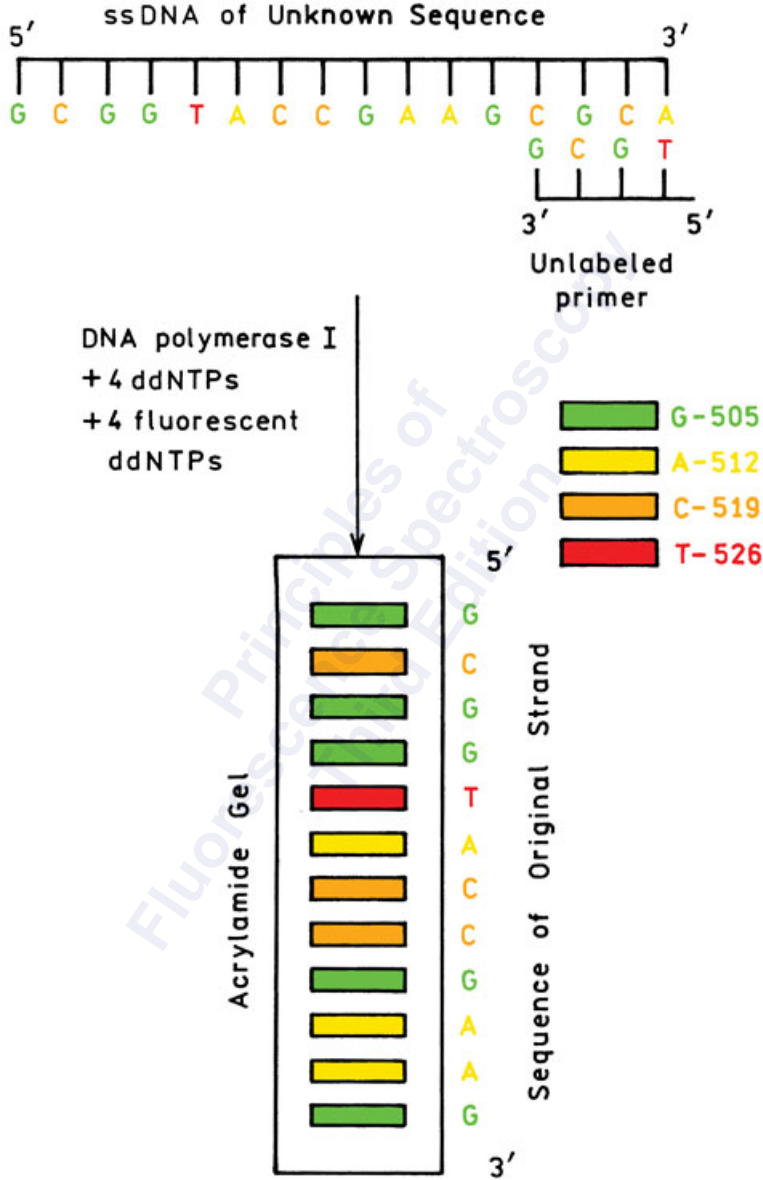
PHASE ANGLE (degrees)  
or MODULATION (%)

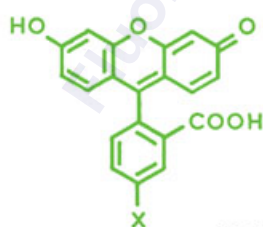
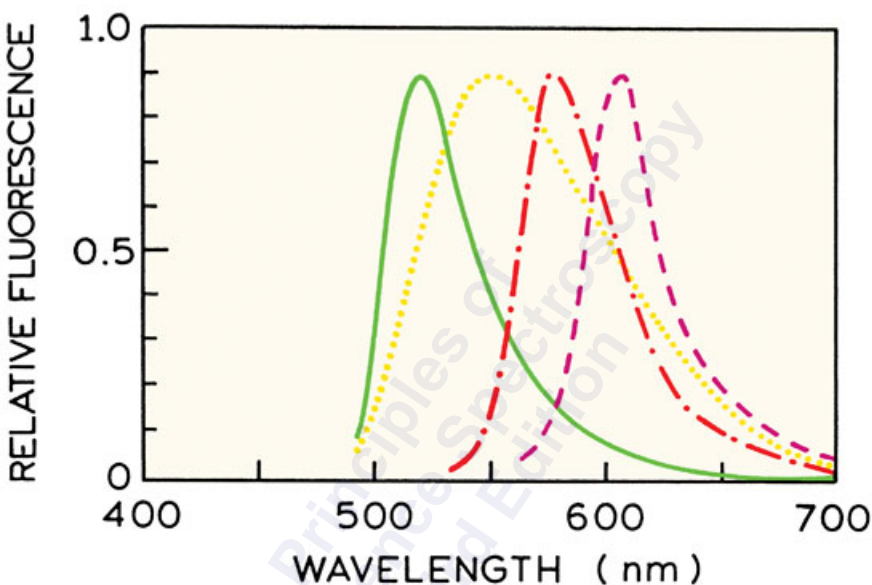
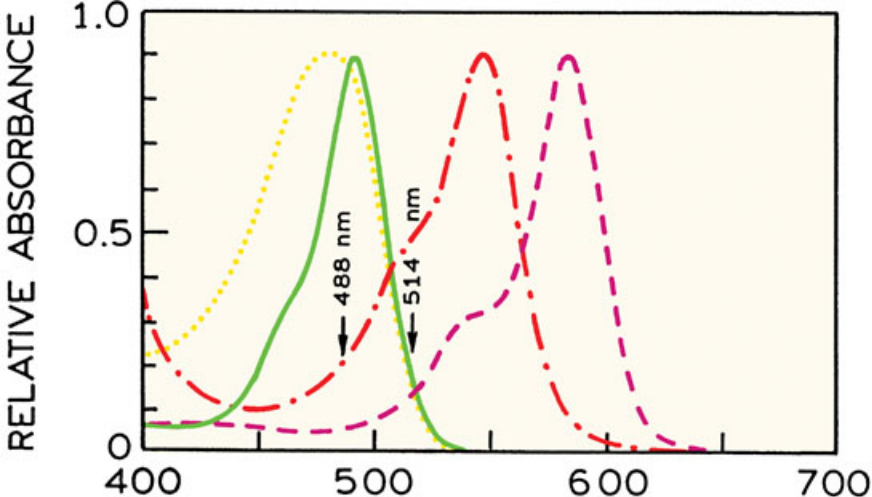






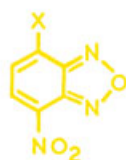






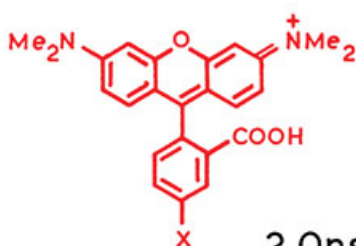
3.3ns

Fluorescein



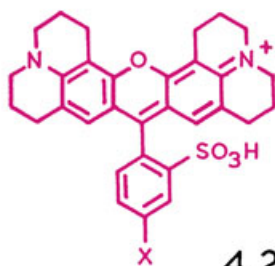
1.1 ns

NBD



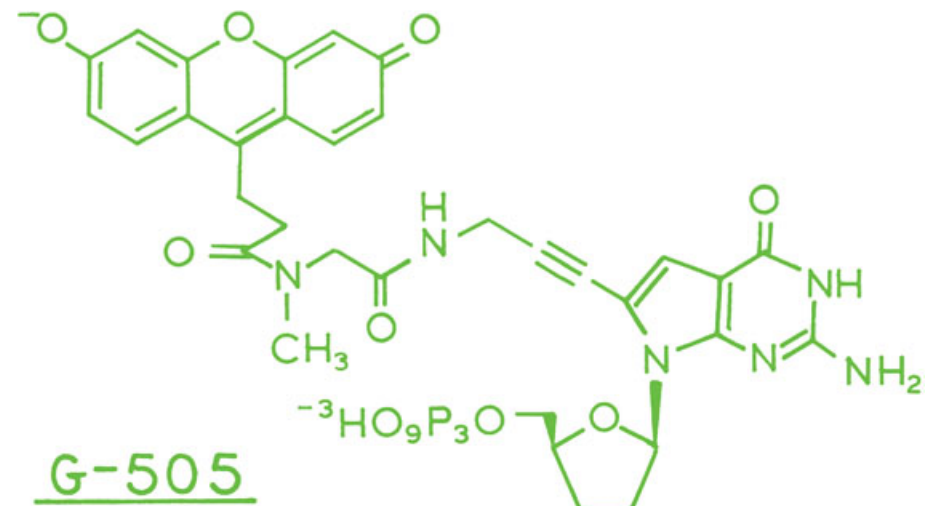
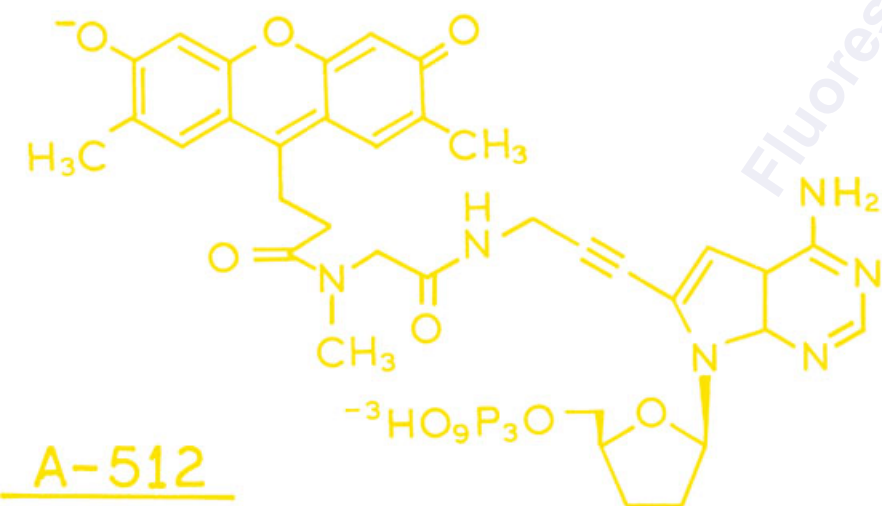
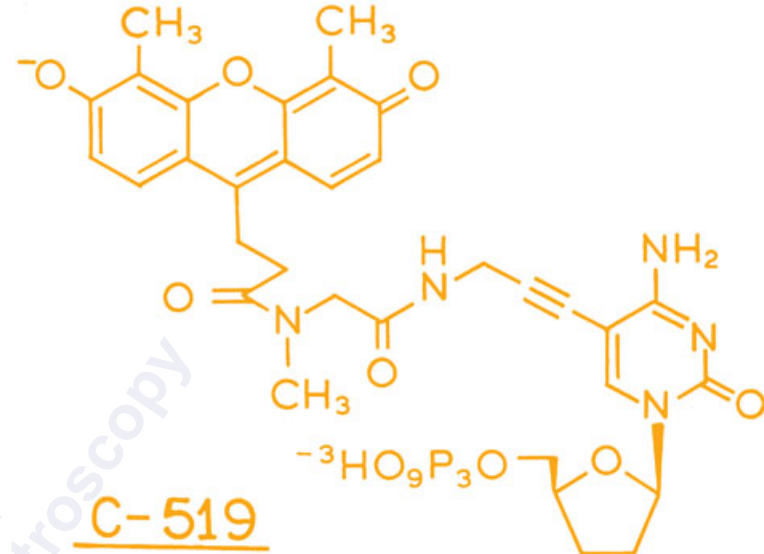
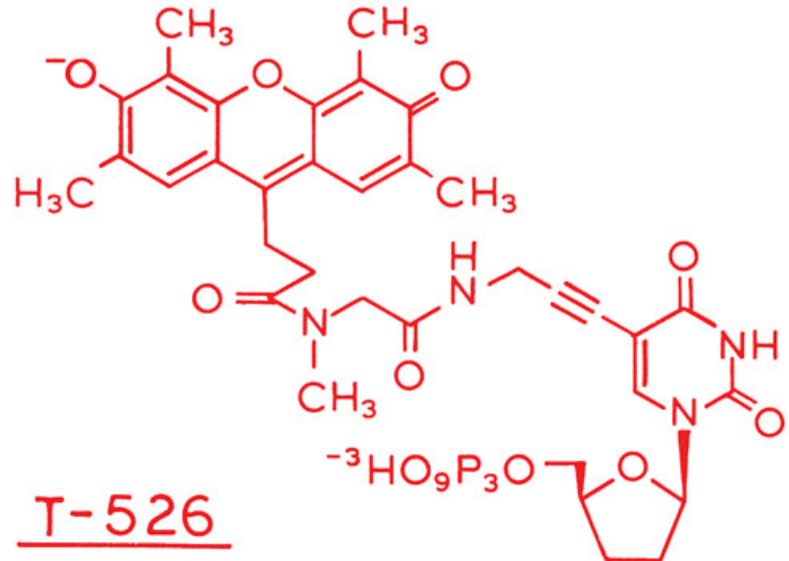
2.0ns

Tetramethylrhodamine



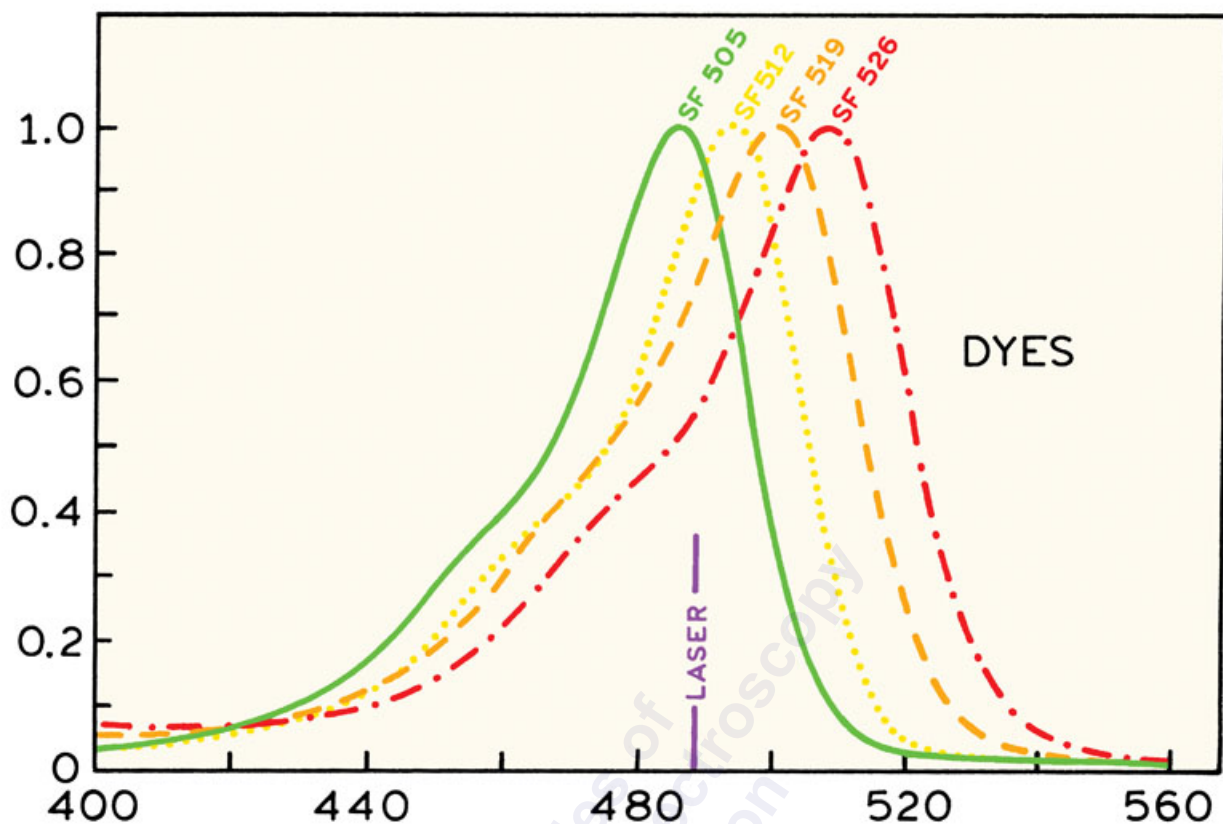
4.2ns

Texas Red

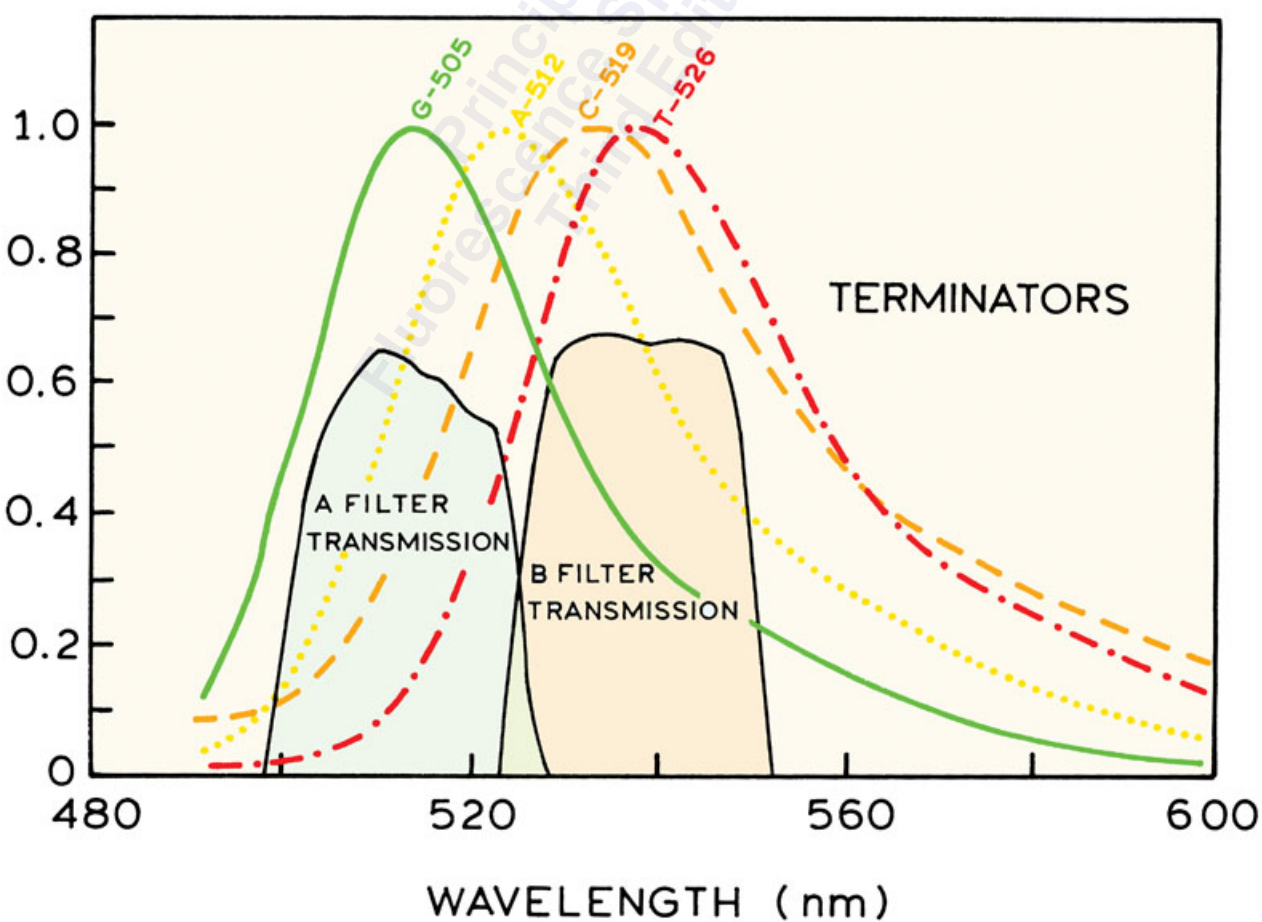


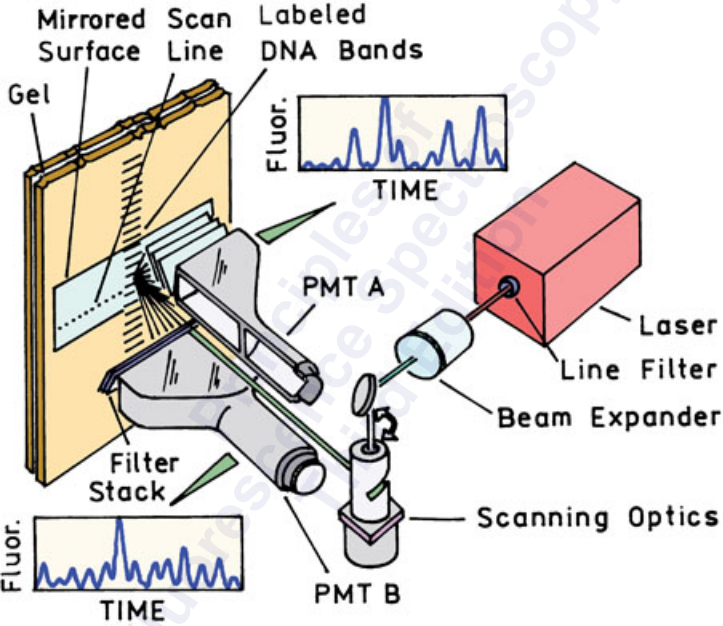
Principles of  
Fluorescence Spectroscopy  
Third Edition

ABSORBANCE

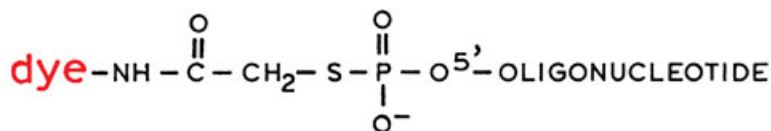
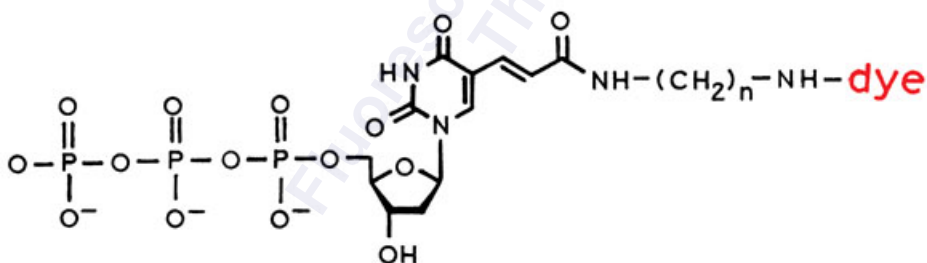
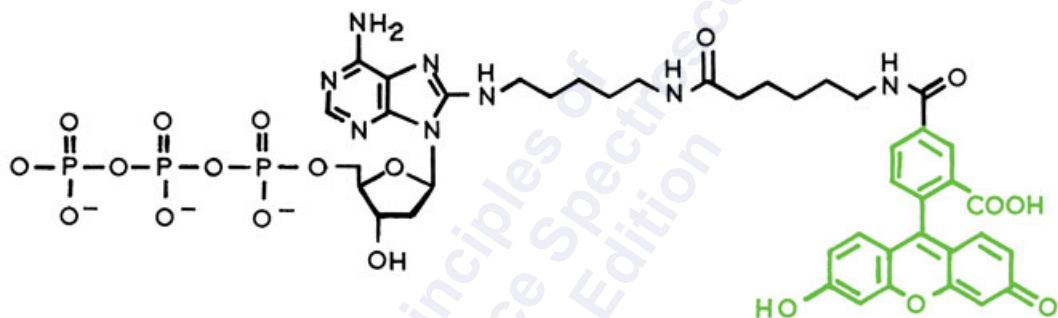
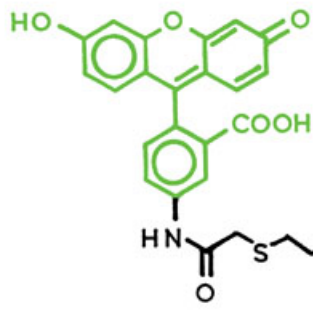


FLUORESCENCE

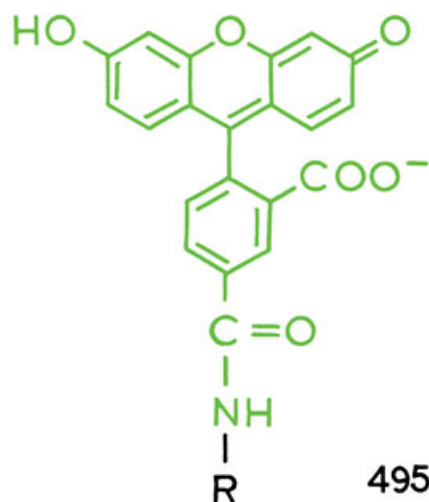








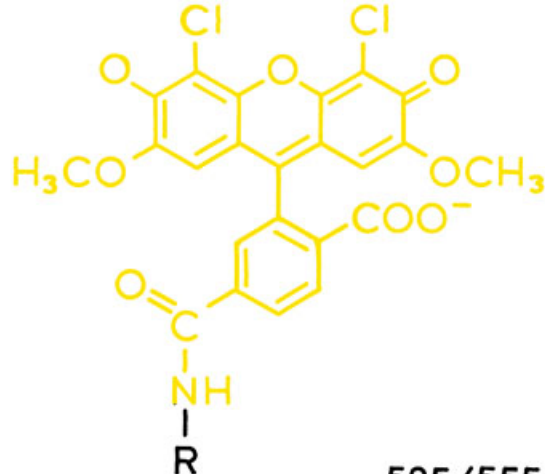




495/525 nm

FAM or F

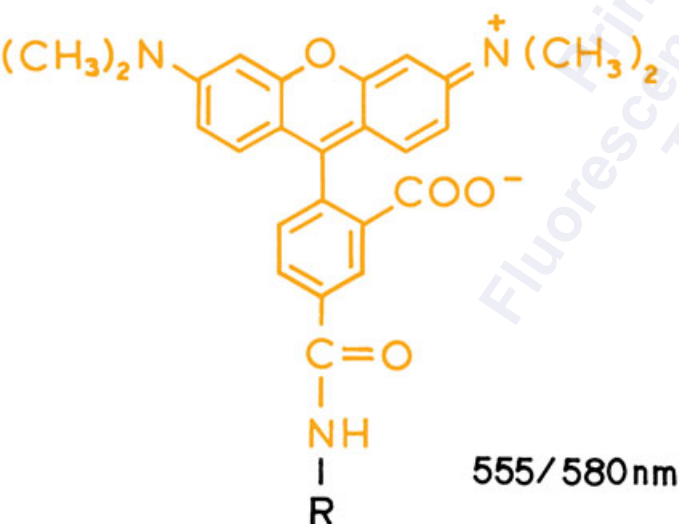
5-carboxyfluorescein



525/555 nm

JOE or J

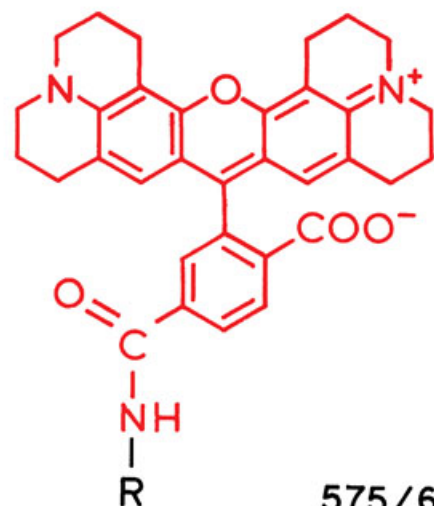
2',7'-dimethoxy-4',5'-  
dichloro-6-carboxy-  
fluorescein



555/580 nm

TAMRA or T

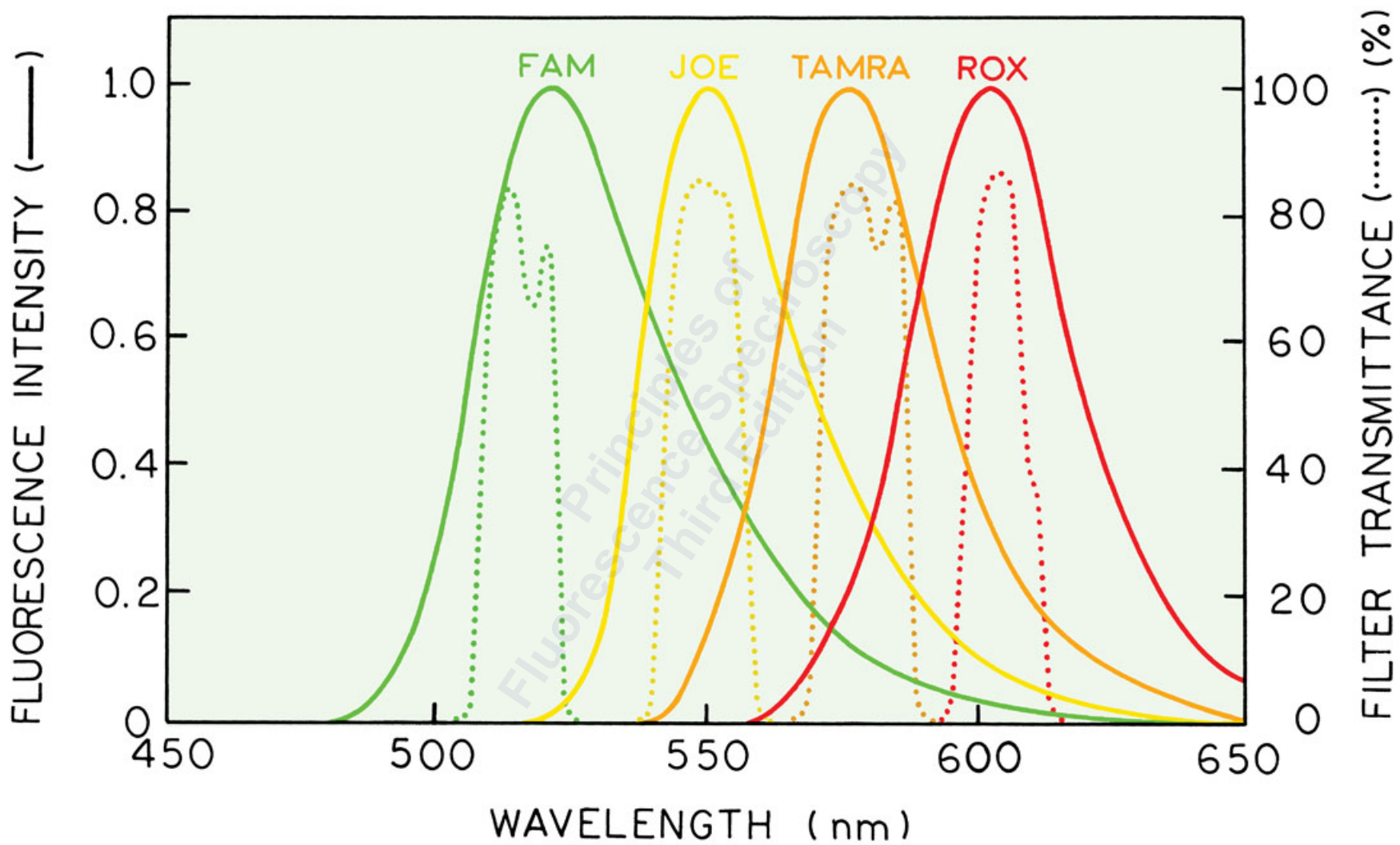
N,N,N',N'-tetramethyl-6-  
carboxyrhodamine

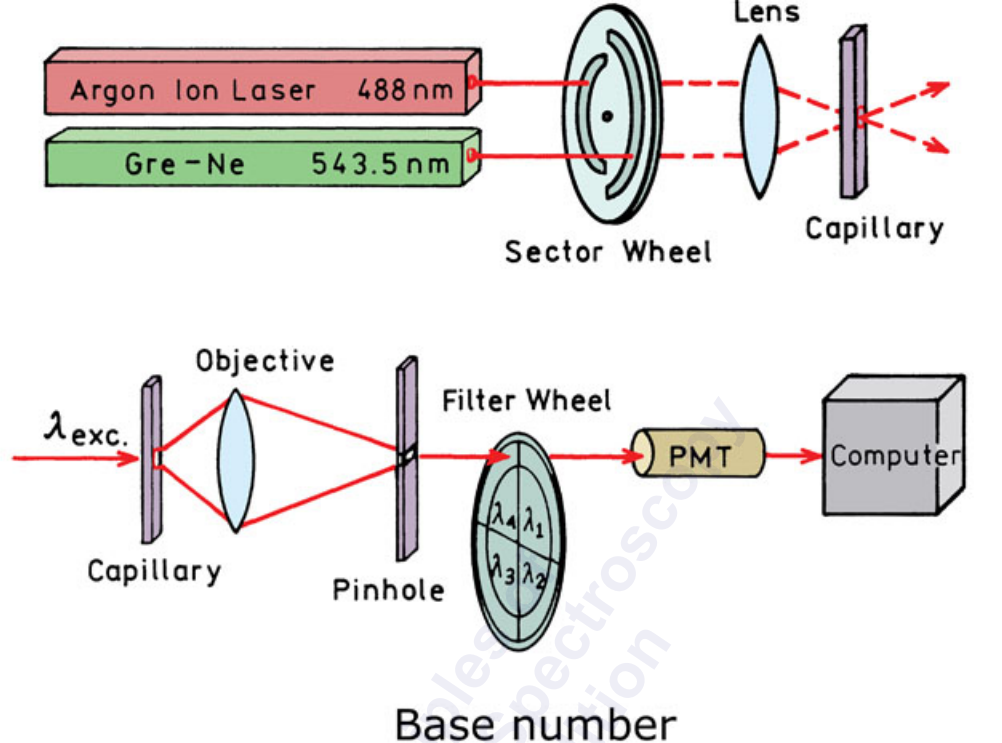


575/602 nm

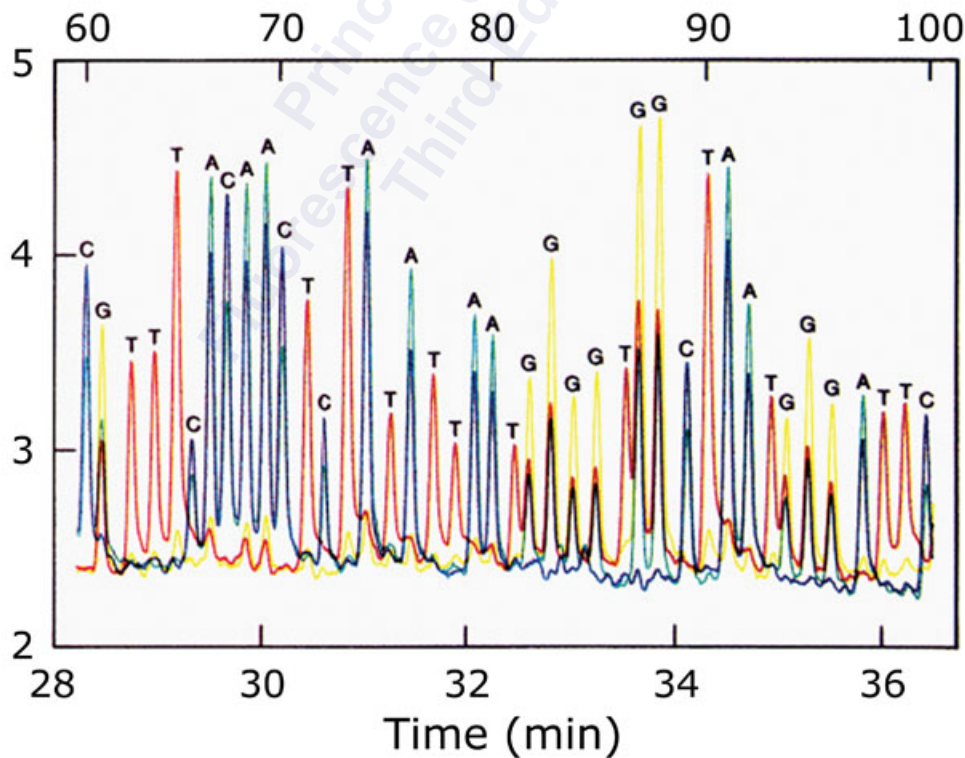
ROX or R

5-carboxy-X-  
rhodamine





Signal (arbitrary units)



Excitation

488 nm

Emission

525 nm



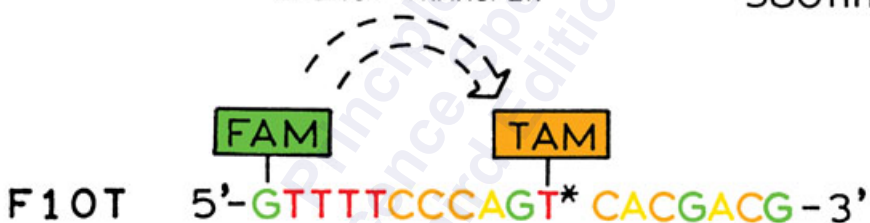
ENERGY TRANSFER

555 nm



ENERGY TRANSFER

580 nm



ENERGY TRANSFER

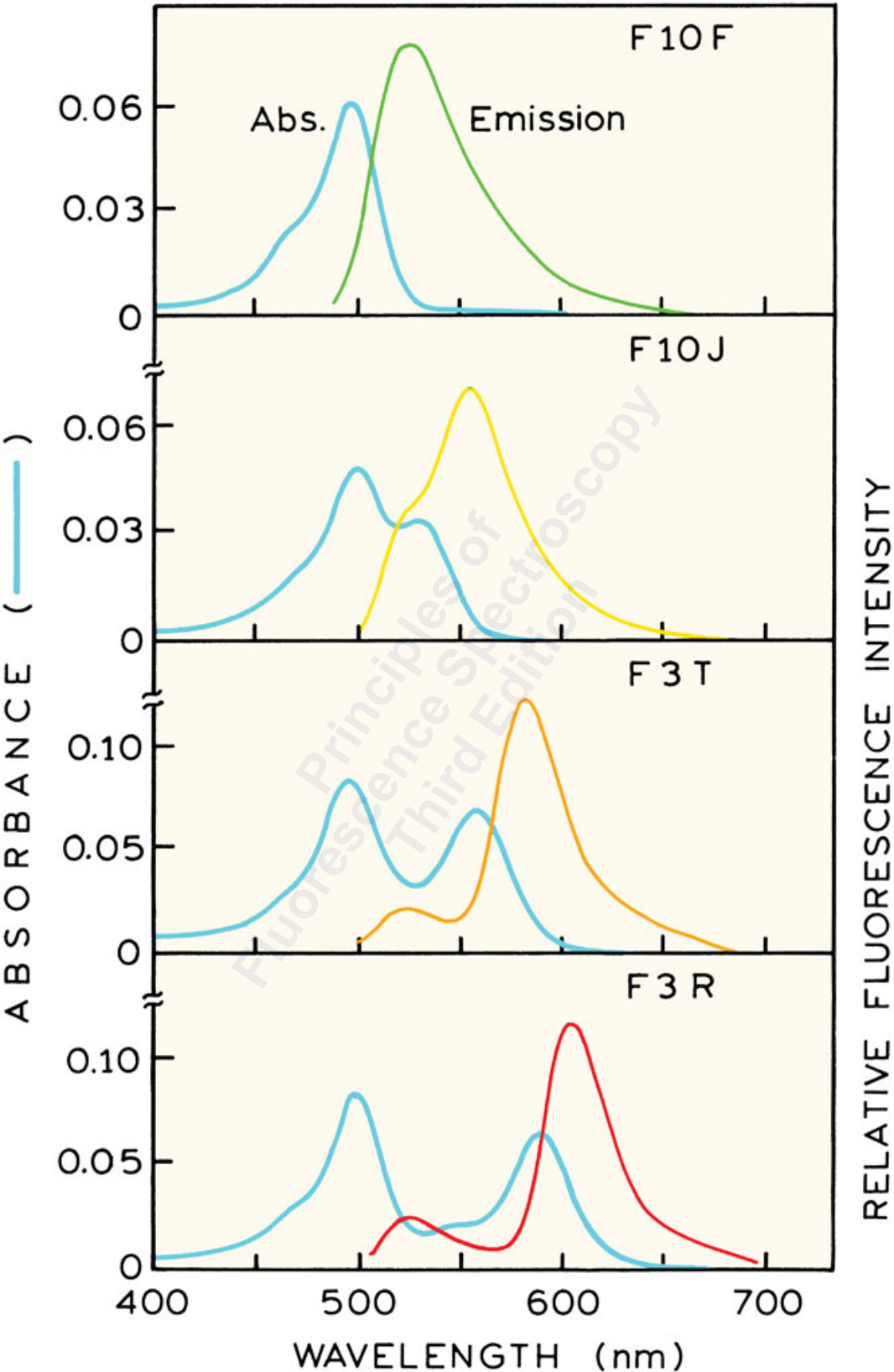
605 nm



Donor      Acceptor



↑  
Desired Primer Sequence

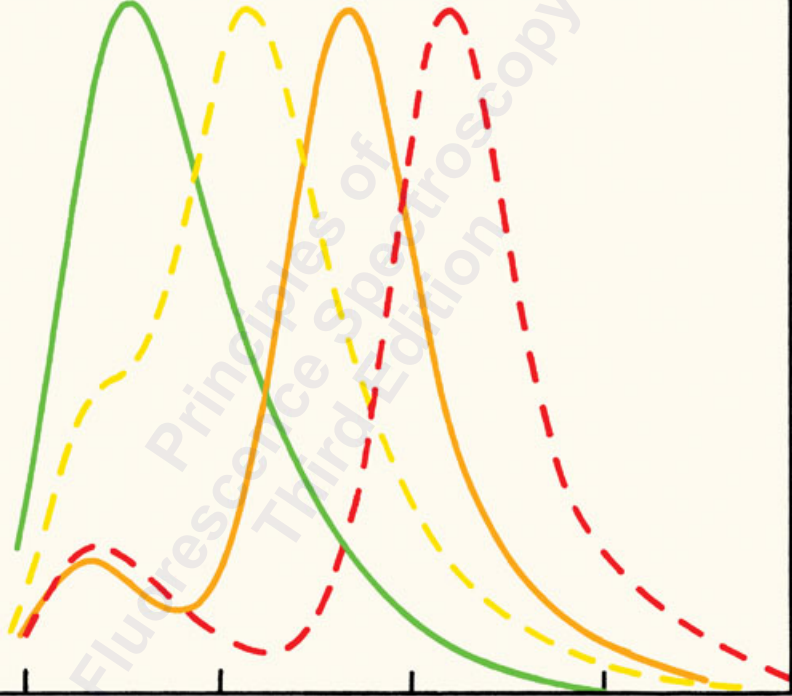




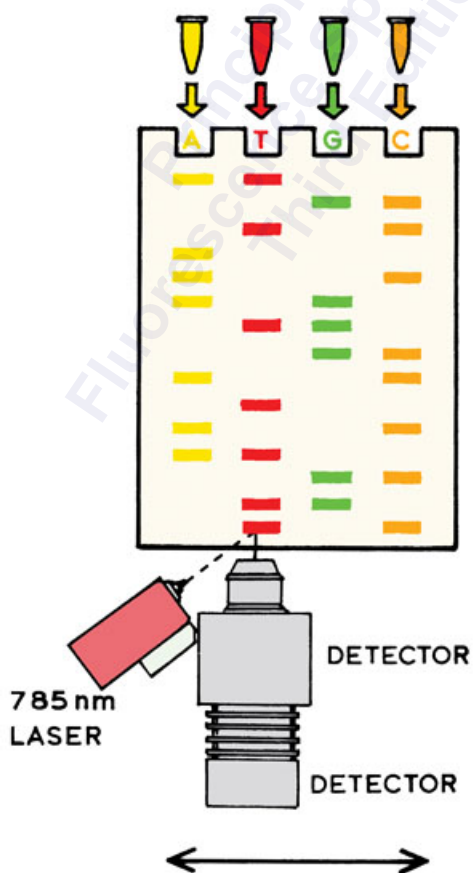
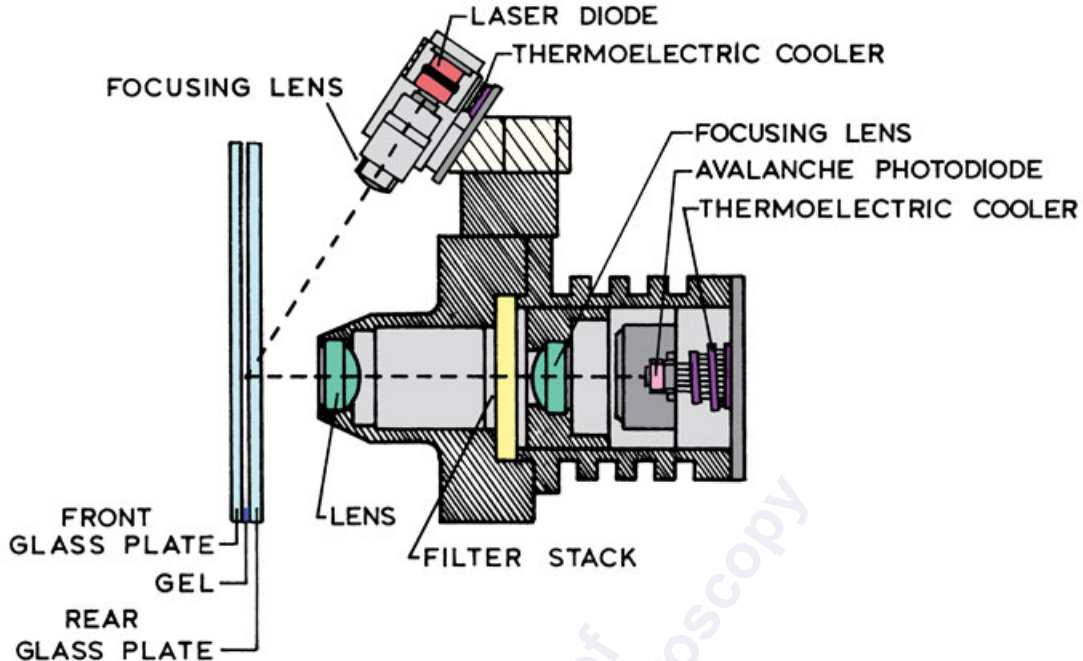
NORMALIZED FLUORESCENCE INT.

F10F F10J F3T F3R

500 550 600 650 700  
WAVELENGTH (nm)









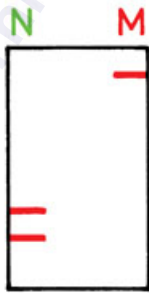


Normal 

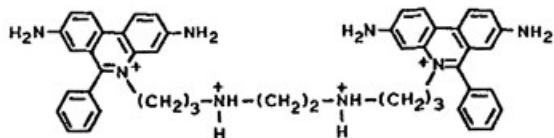
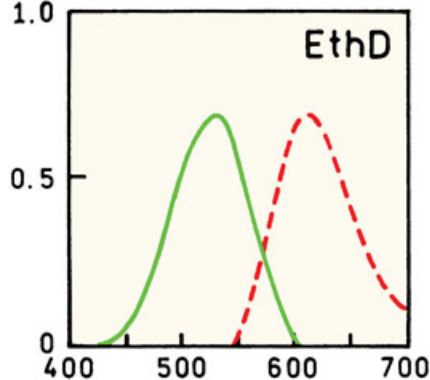
Mutant 

↓  
Restriction digest of DNA

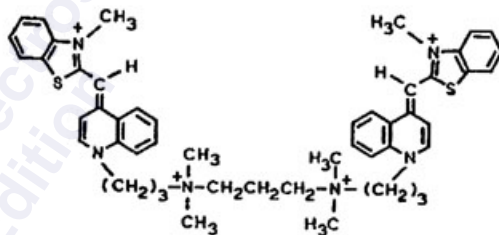
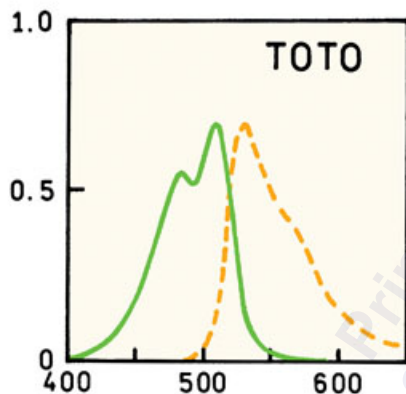
↓  
Electrophoresis



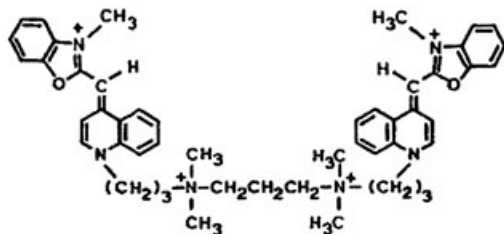
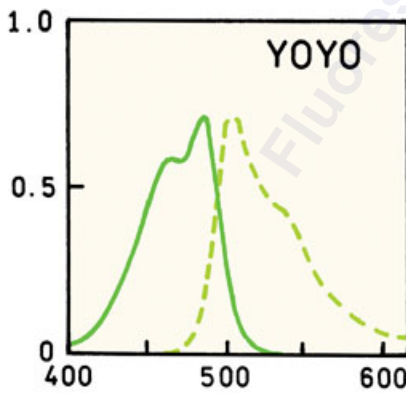
ABSORPTION or FLUORESCENCE



**EthD**

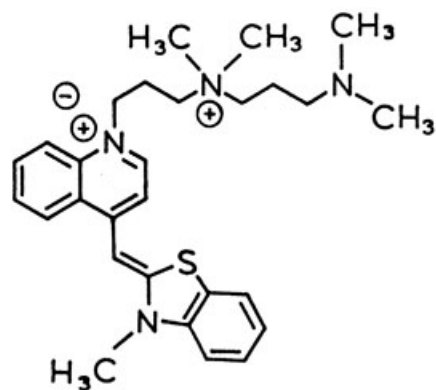


**TOTO**

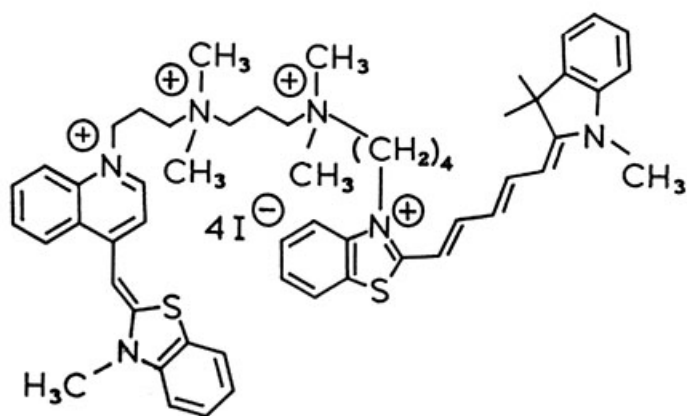


**YOYO**

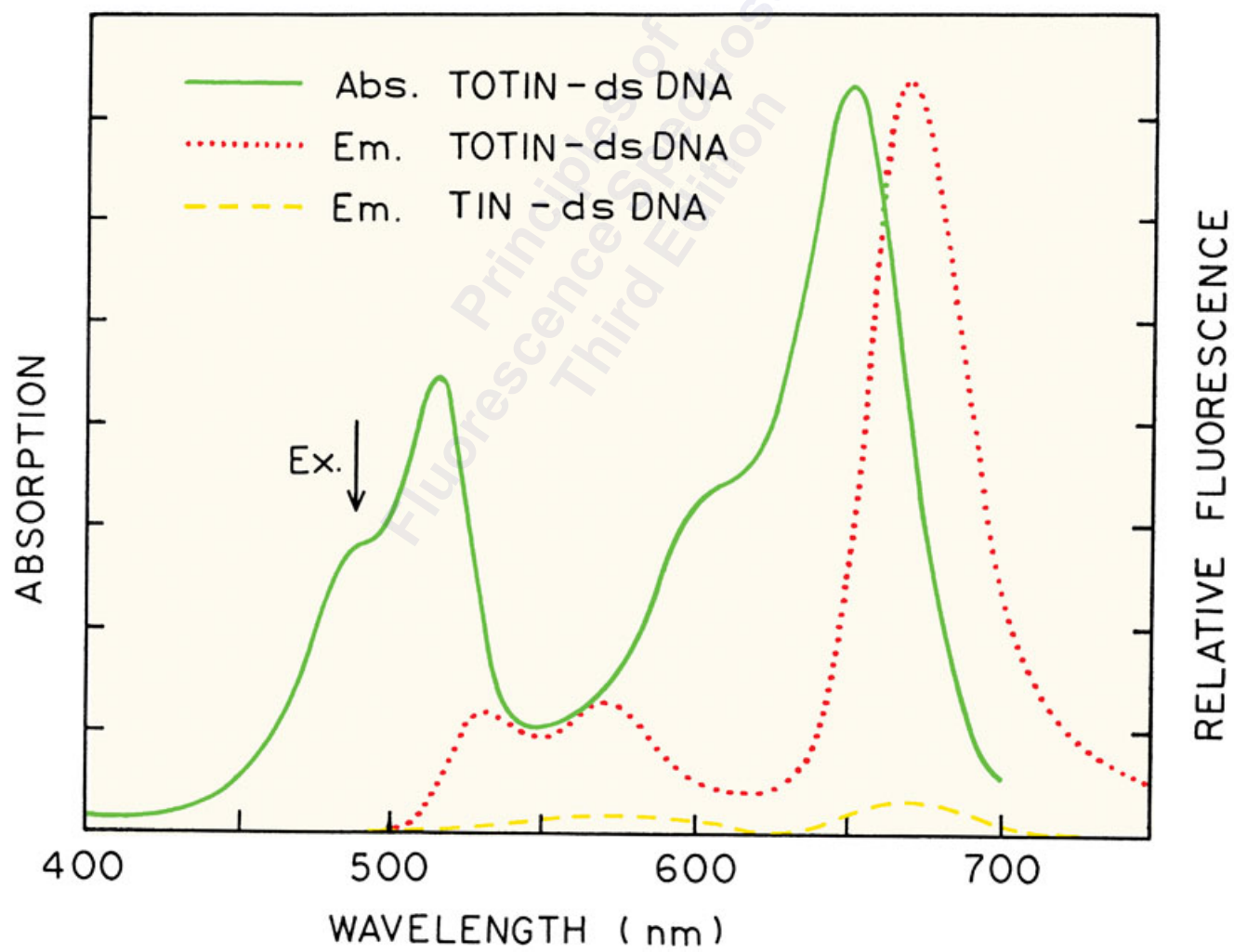
WAVELENGTH (nm)

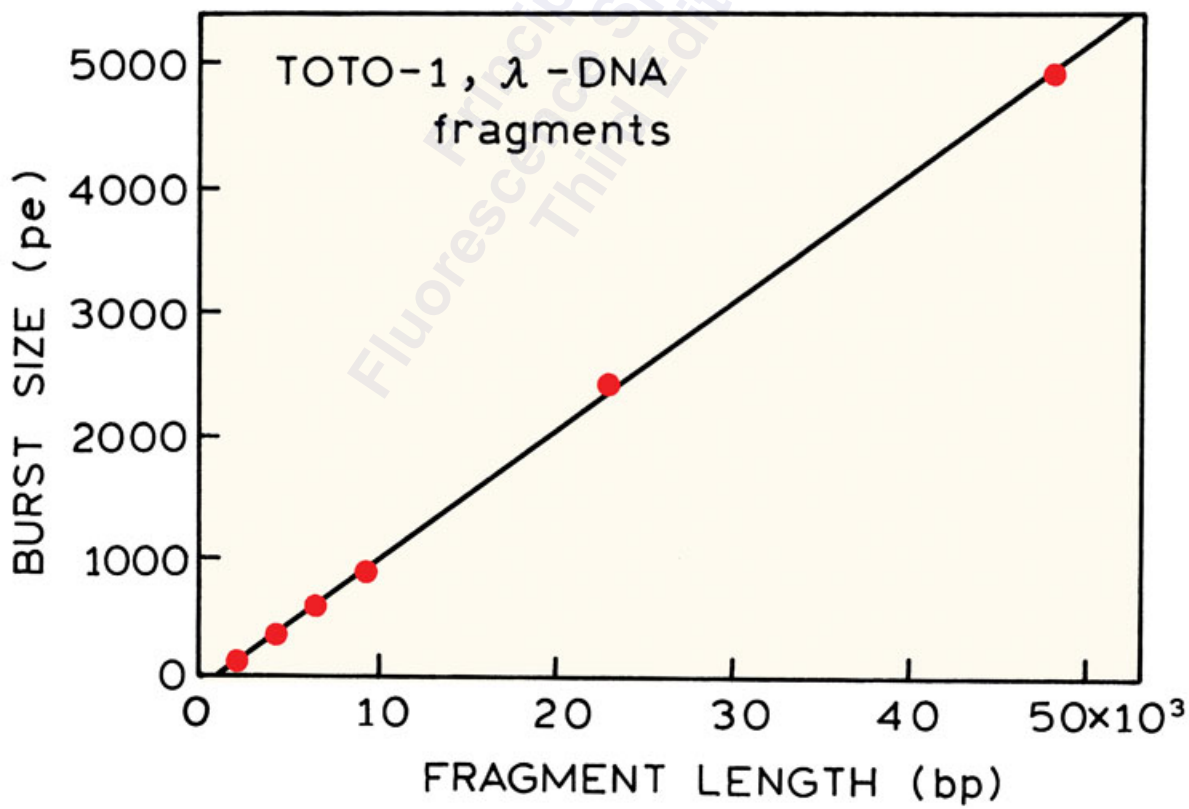
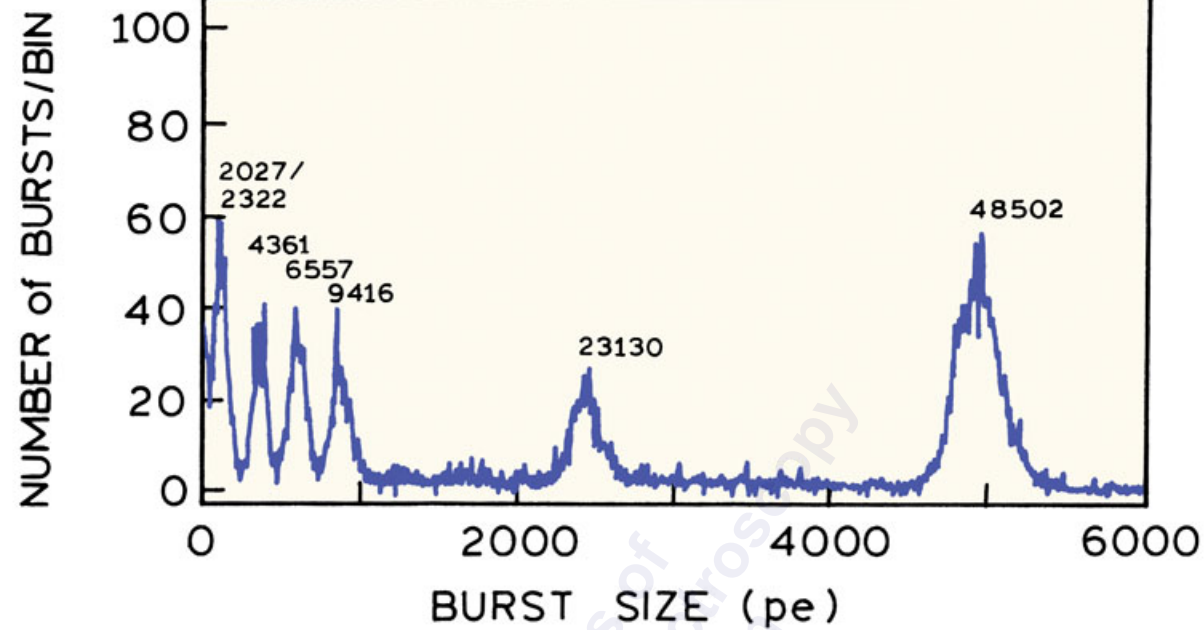


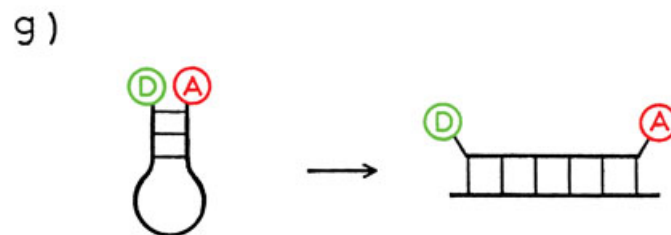
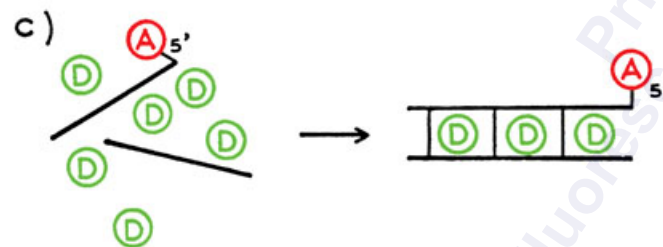
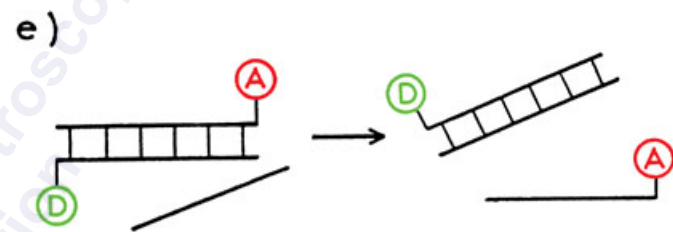
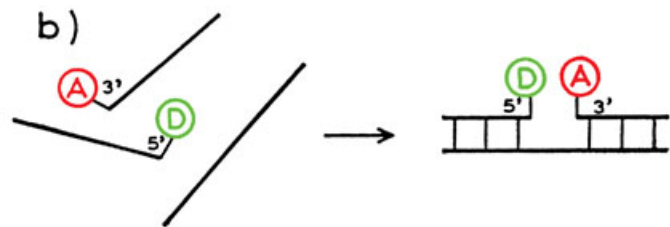
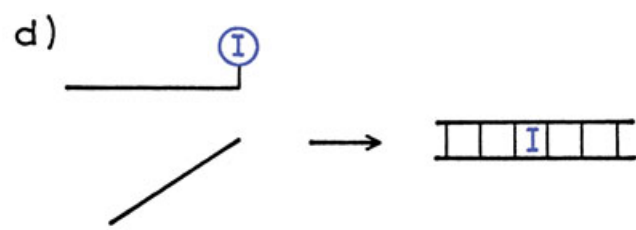
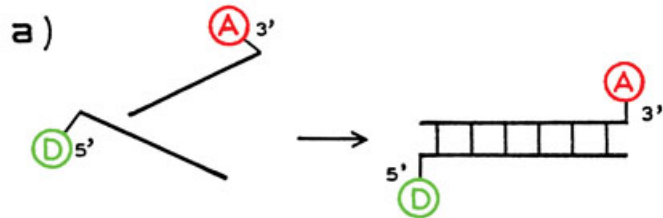
TO



TOTIN

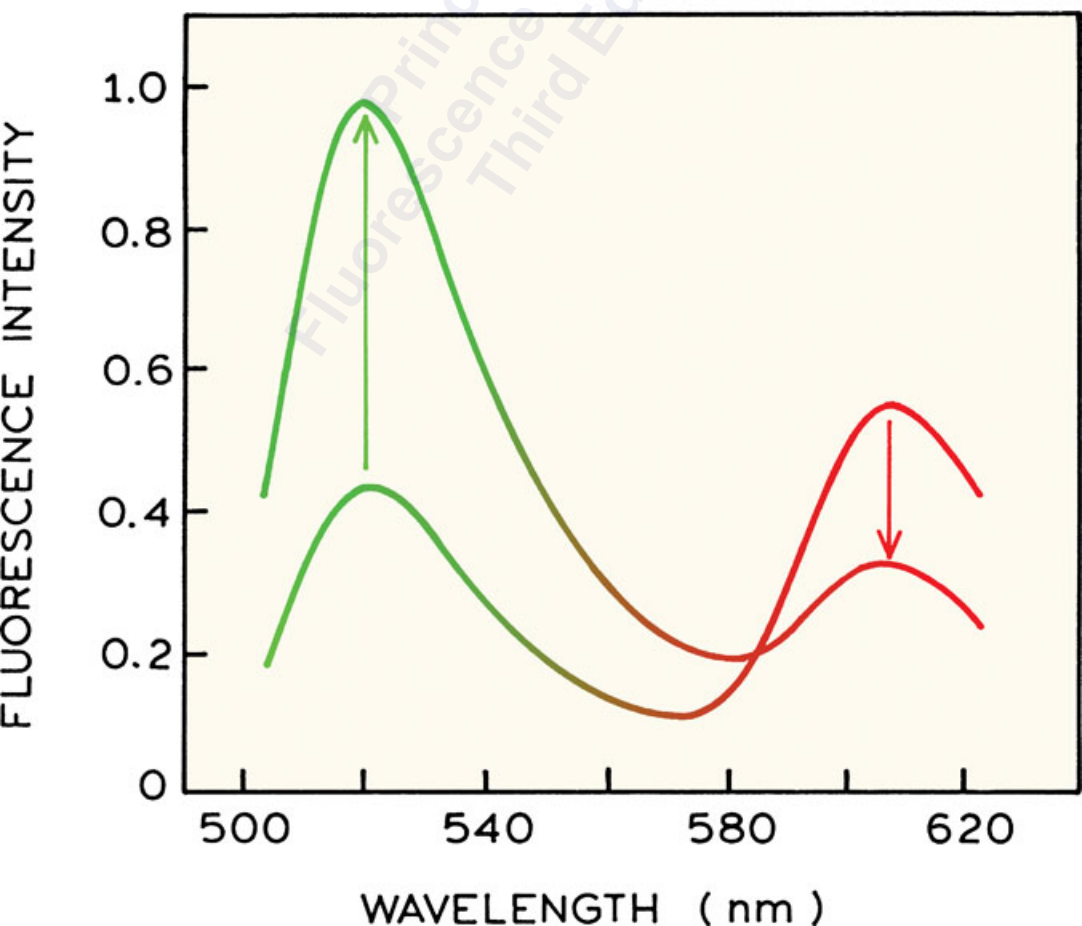
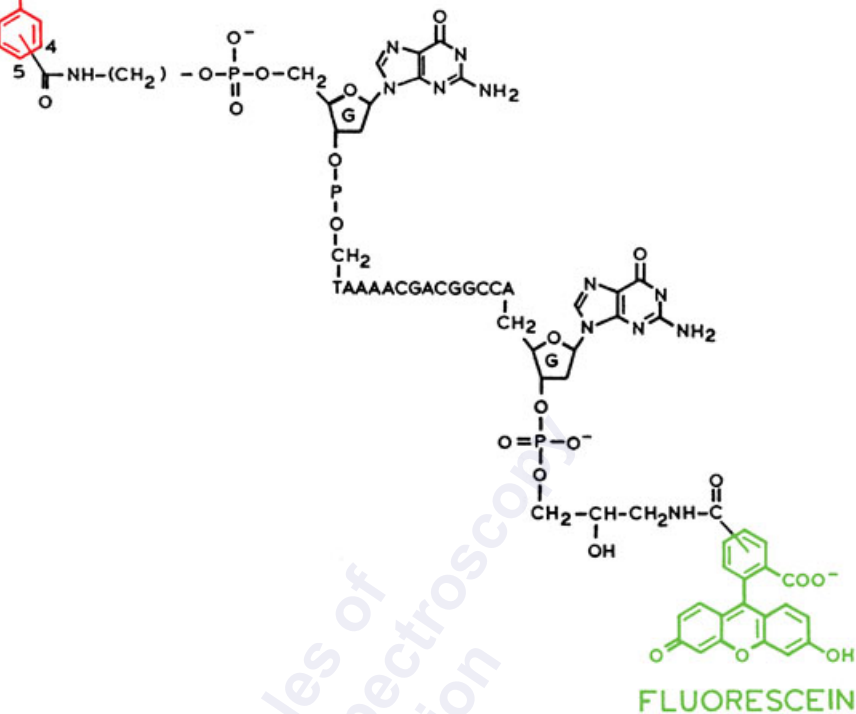




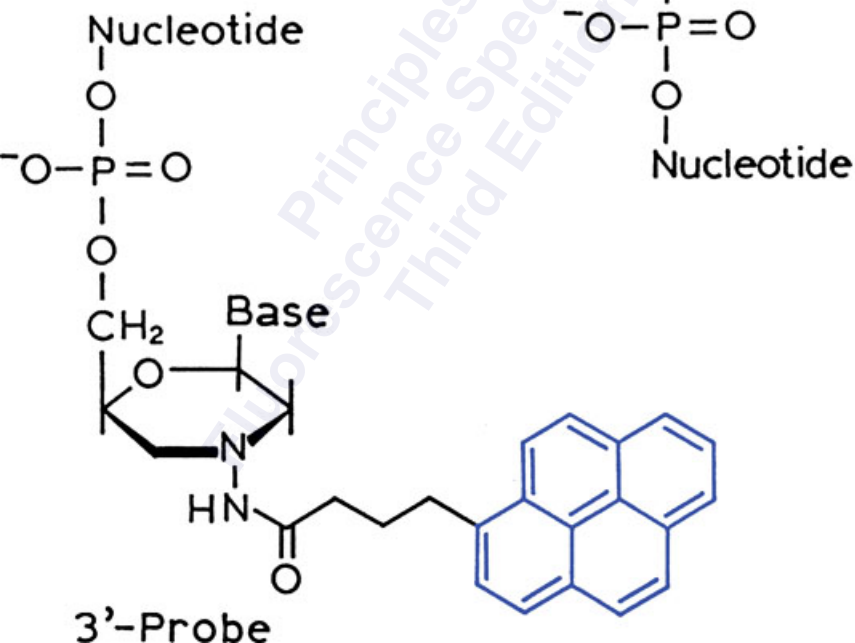
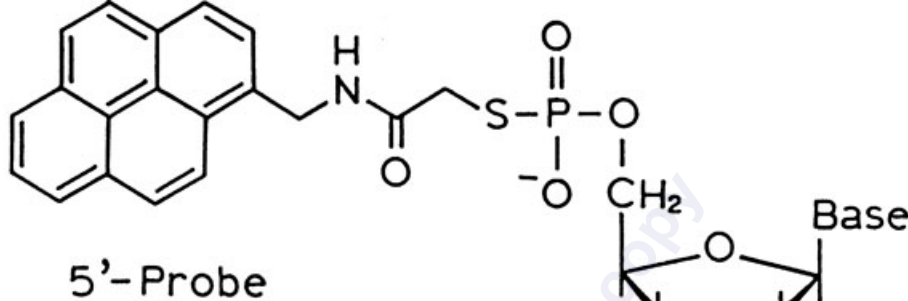




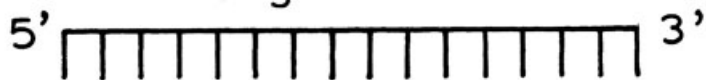






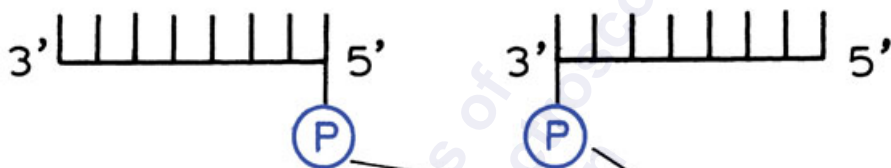


Target 32-mer



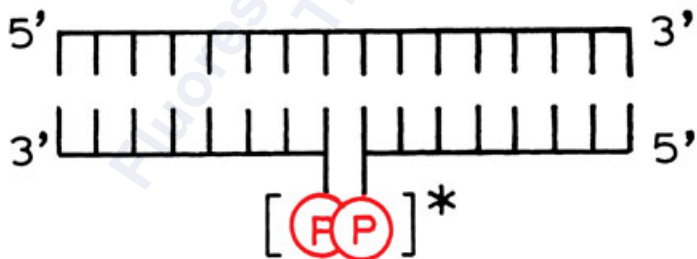
5'-Probe

3'-Probe

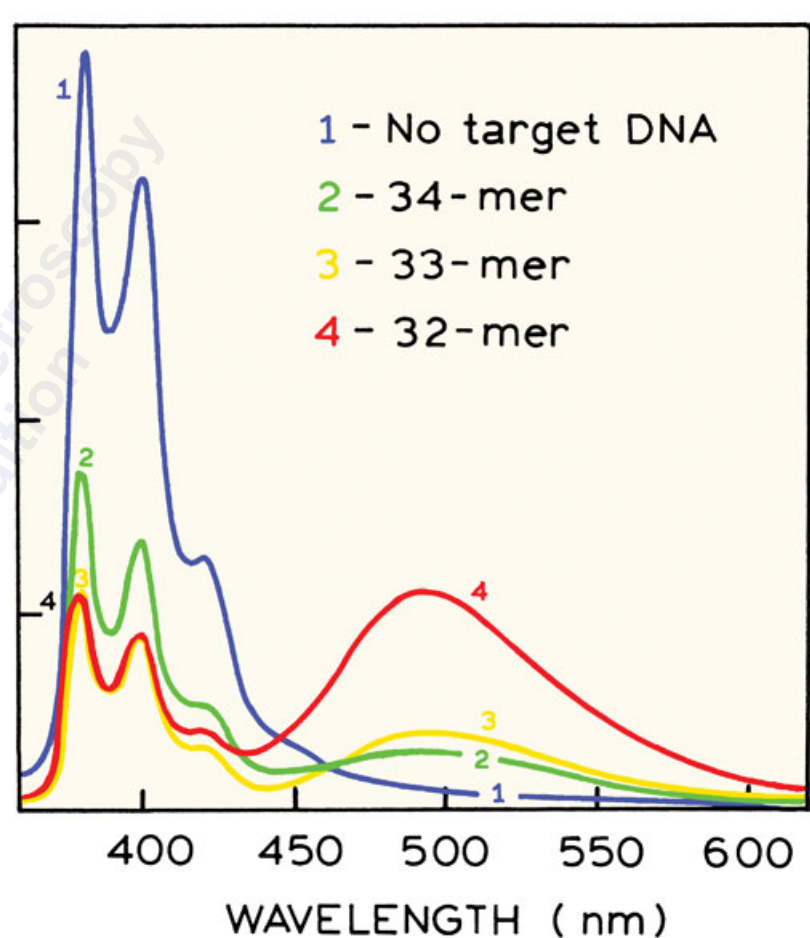
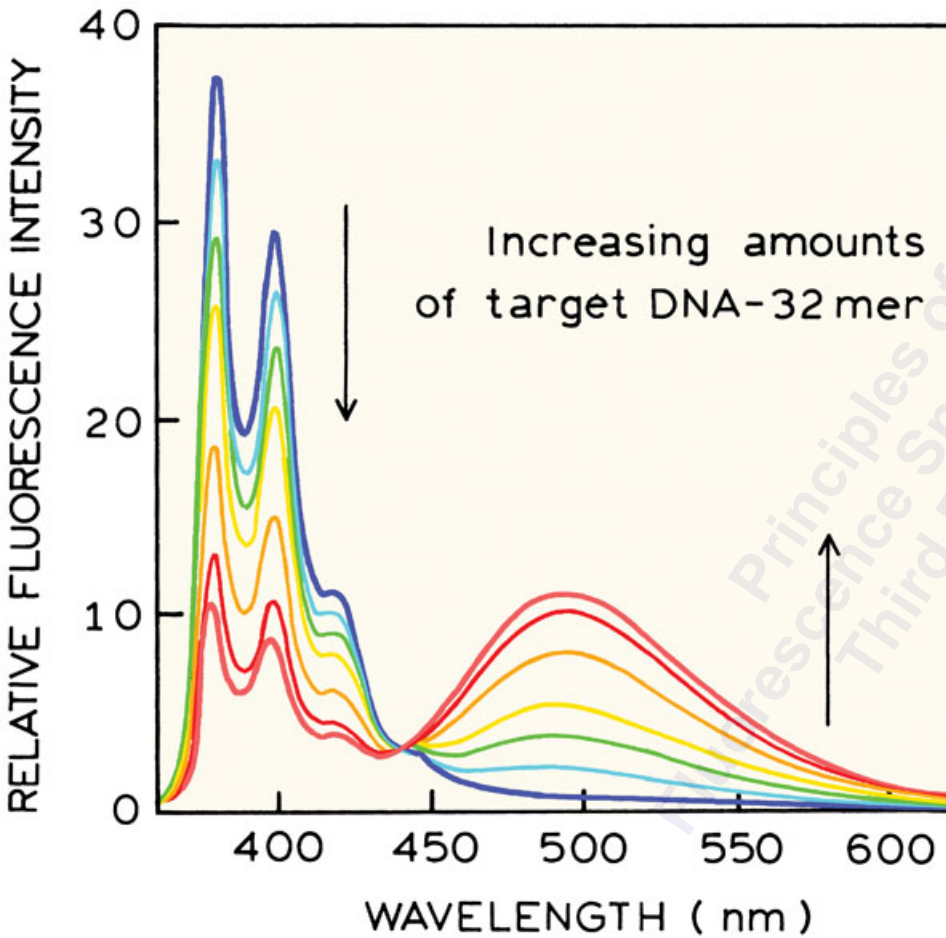


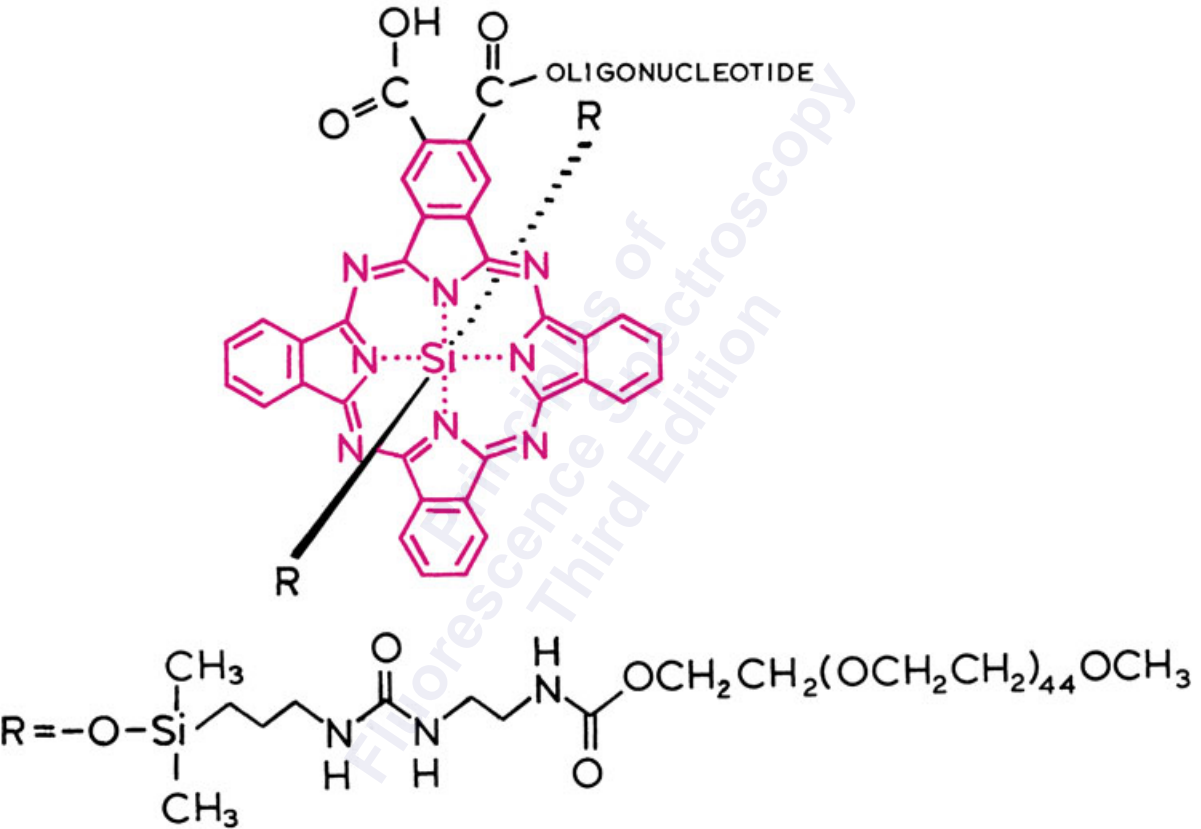
Pyrene

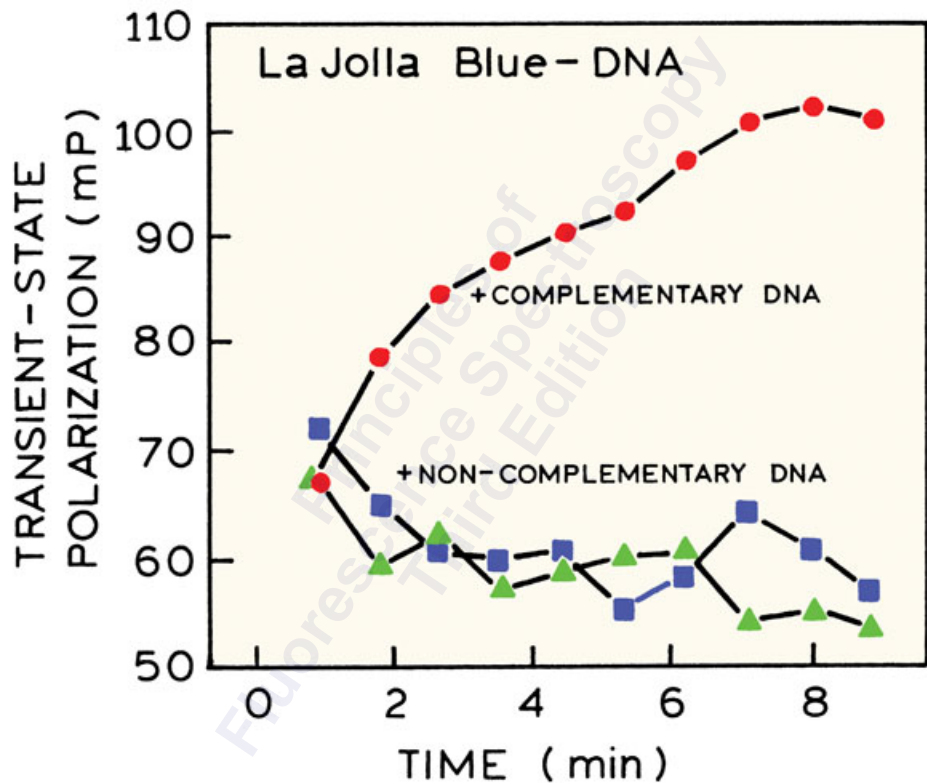
Hybridization



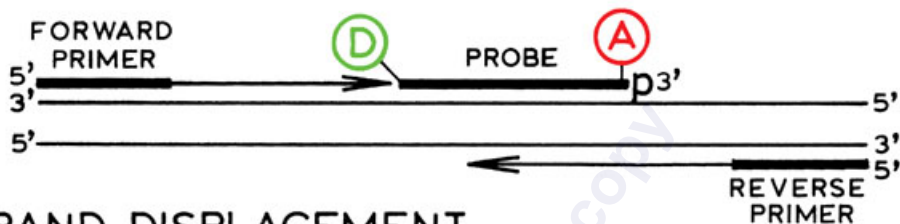
Excimer Emission



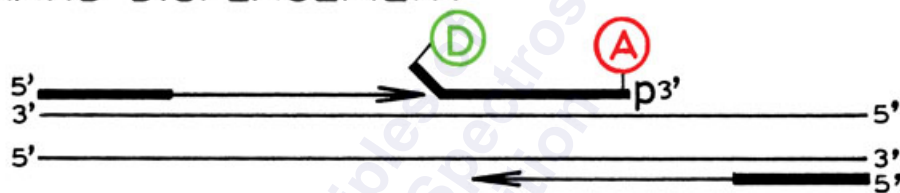




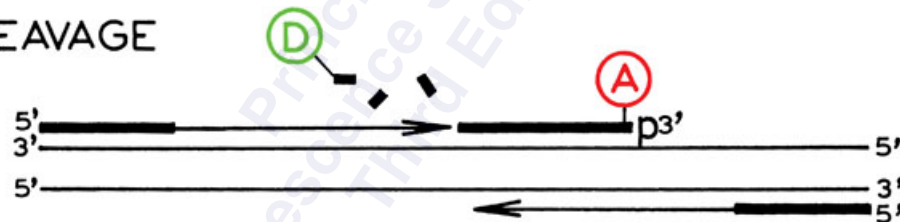
# POLYMERIZATION



## STRAND DISPLACEMENT

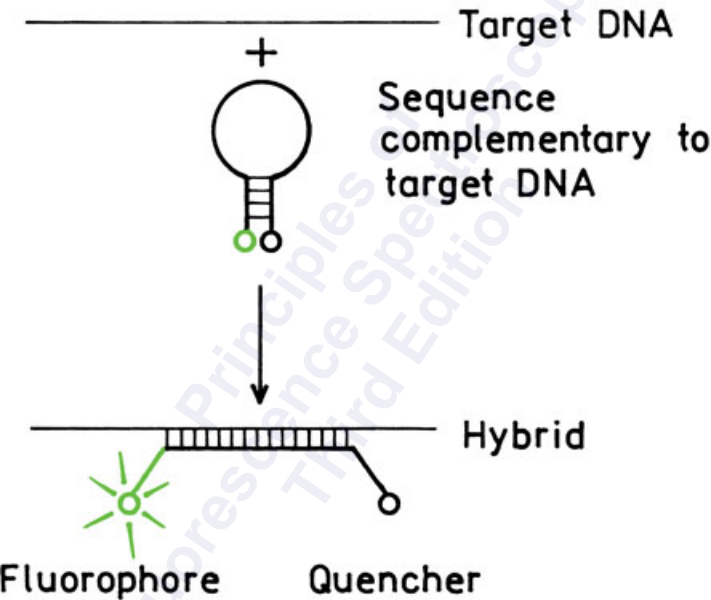


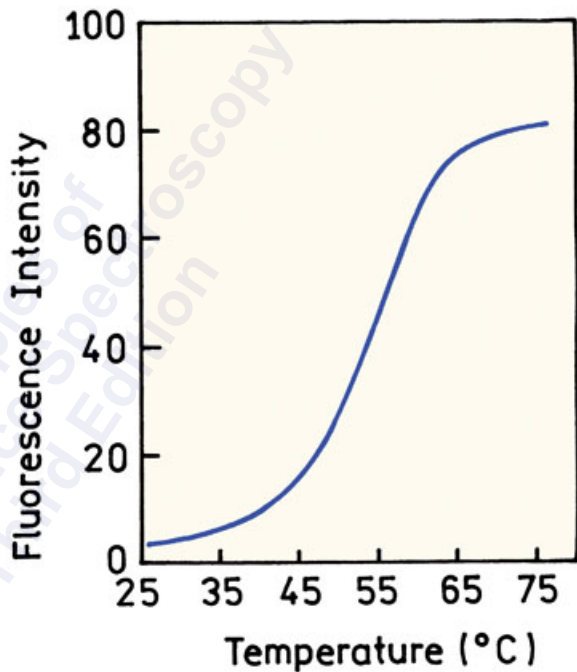
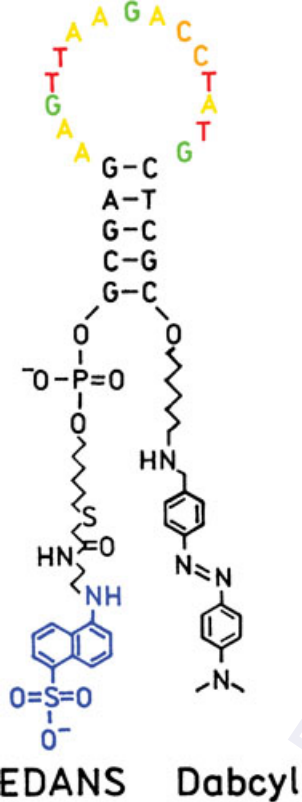
## CLEAVAGE



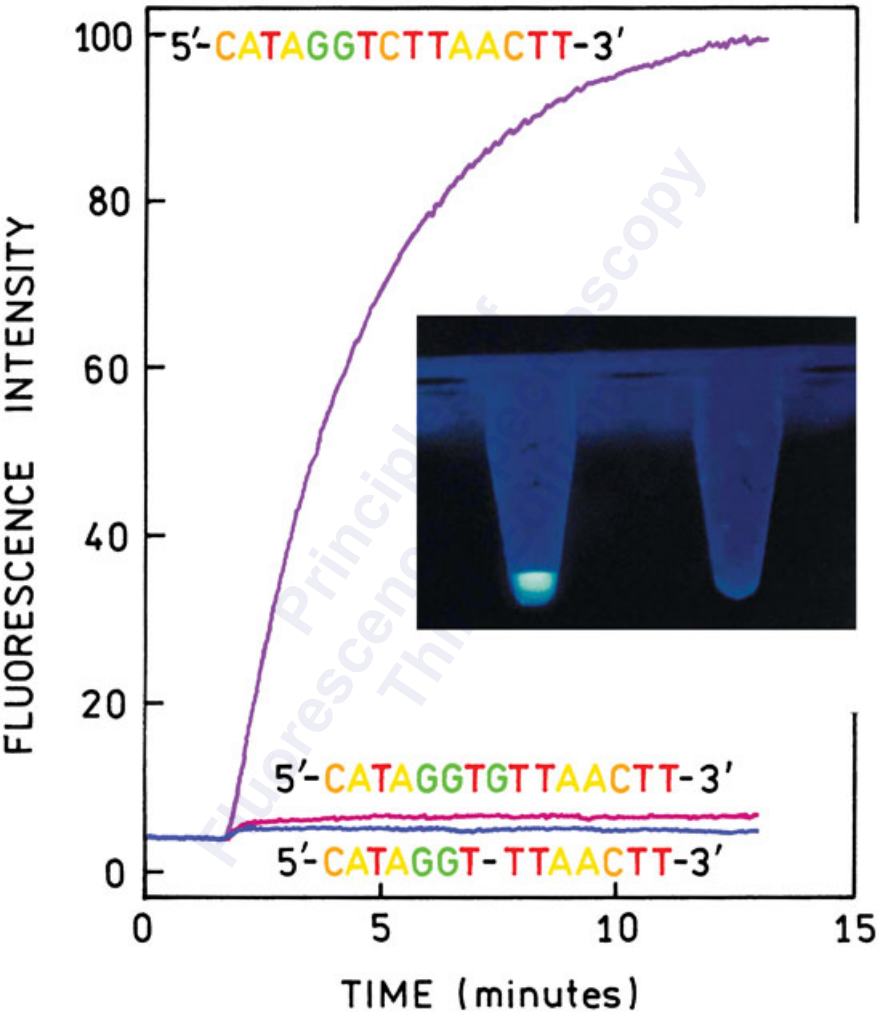
## POLYMERIZATION COMPLETED



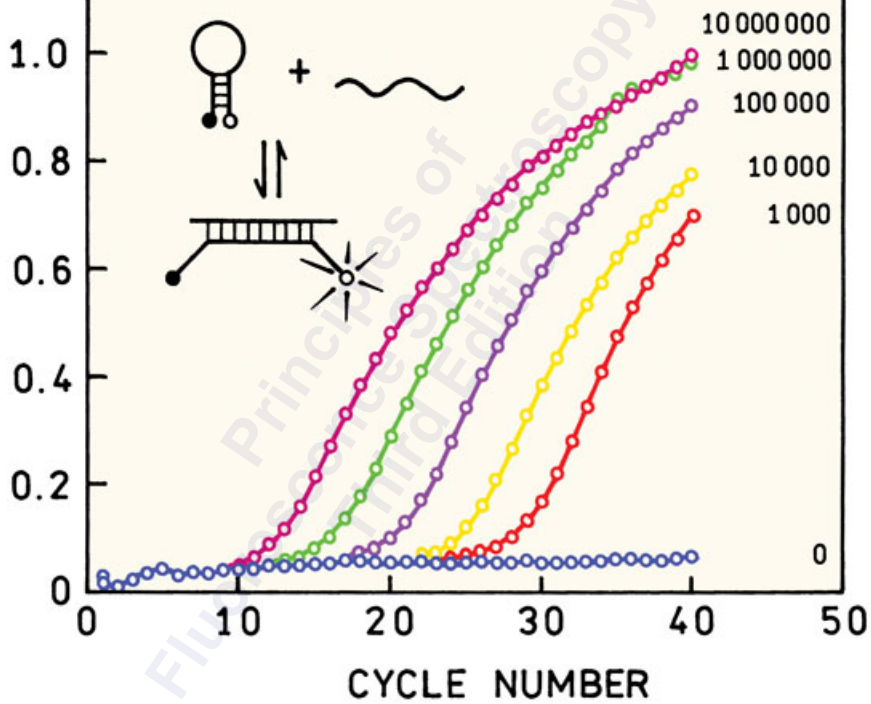








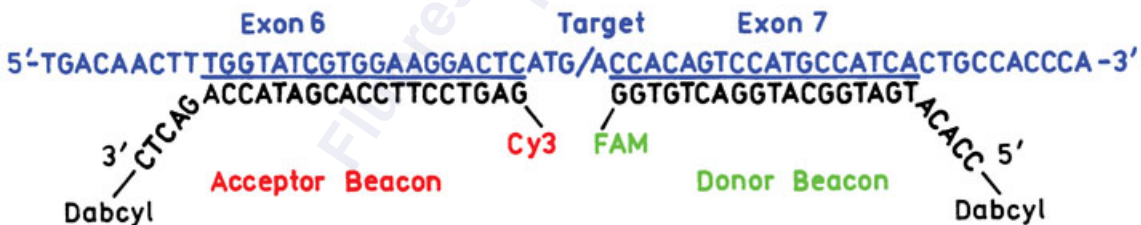
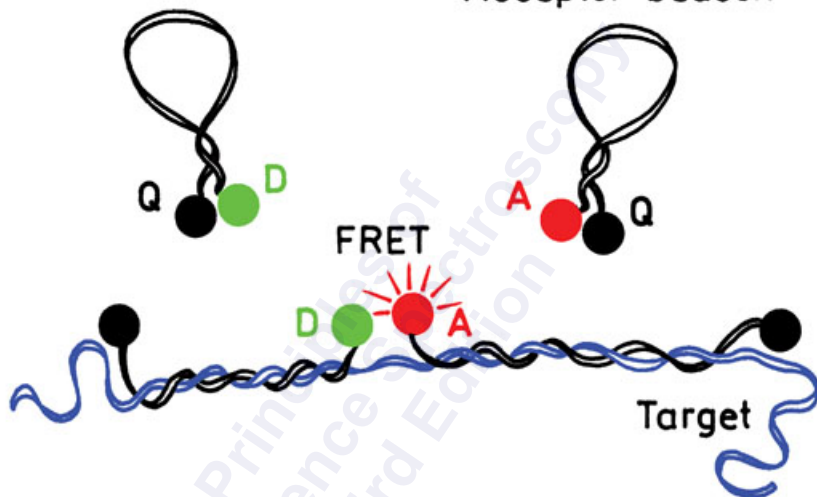
FLUORESCENCE

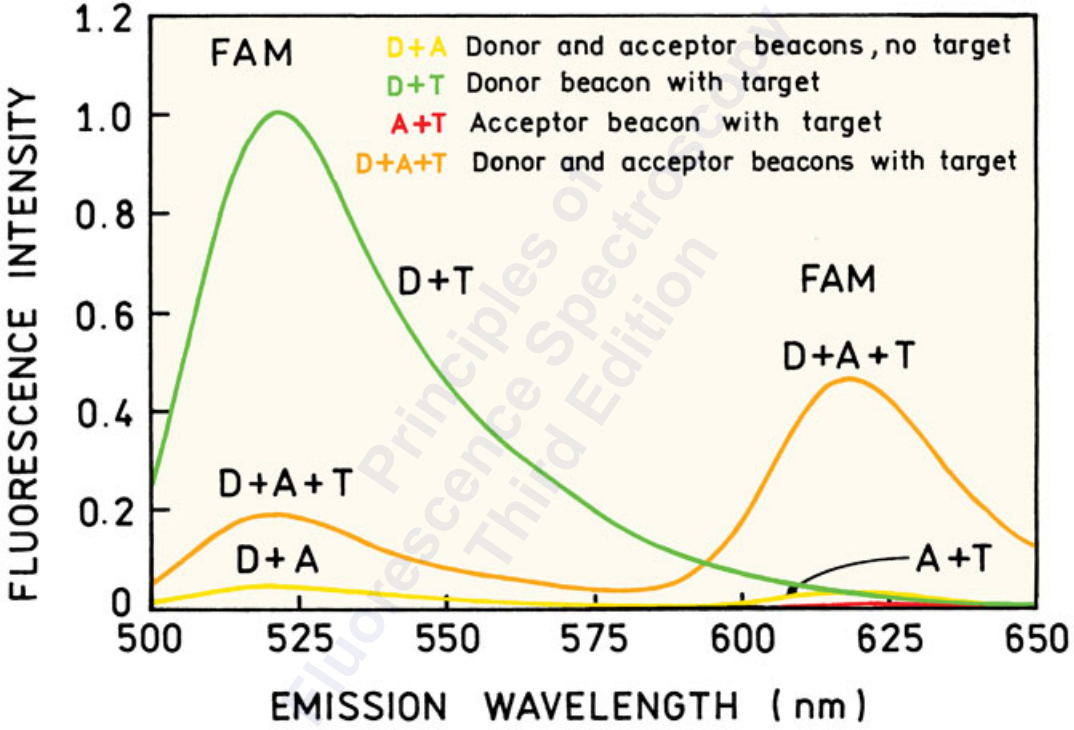


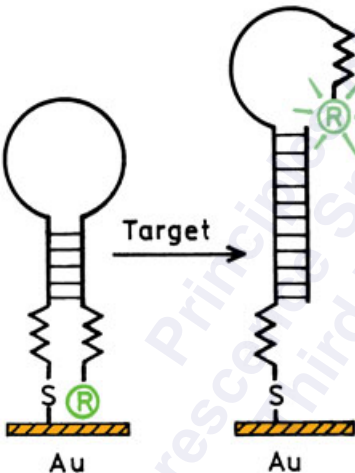


Donor beacon

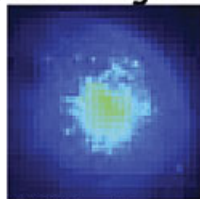
Acceptor beacon



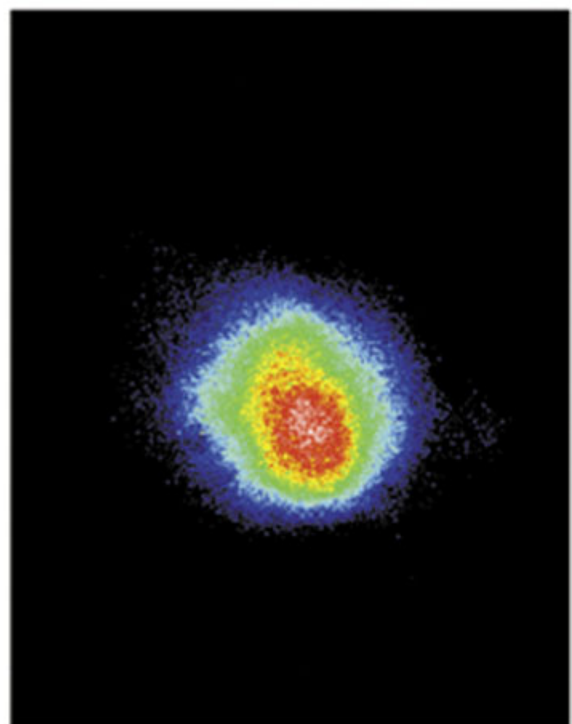
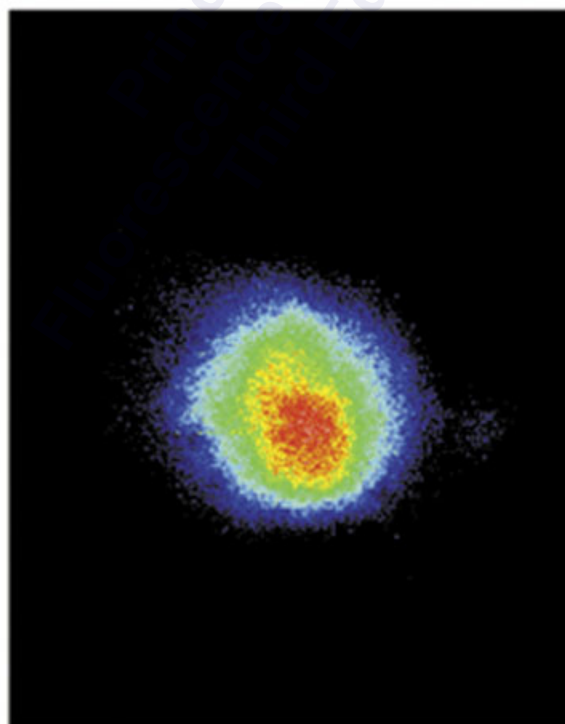
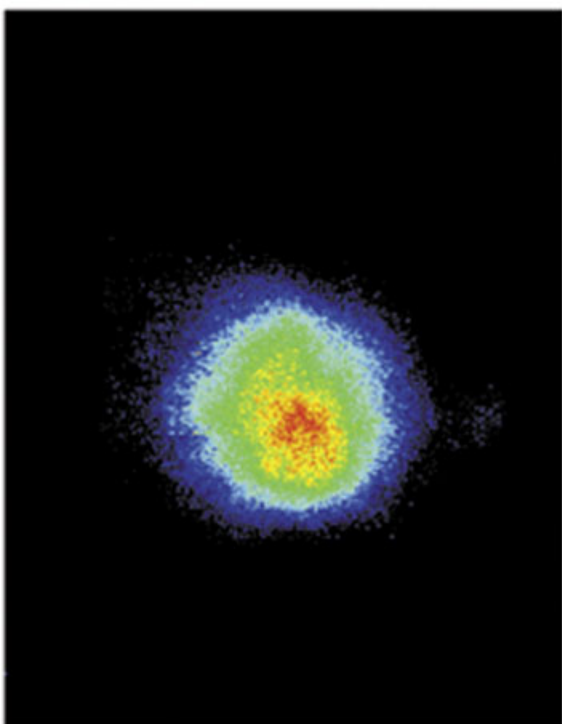
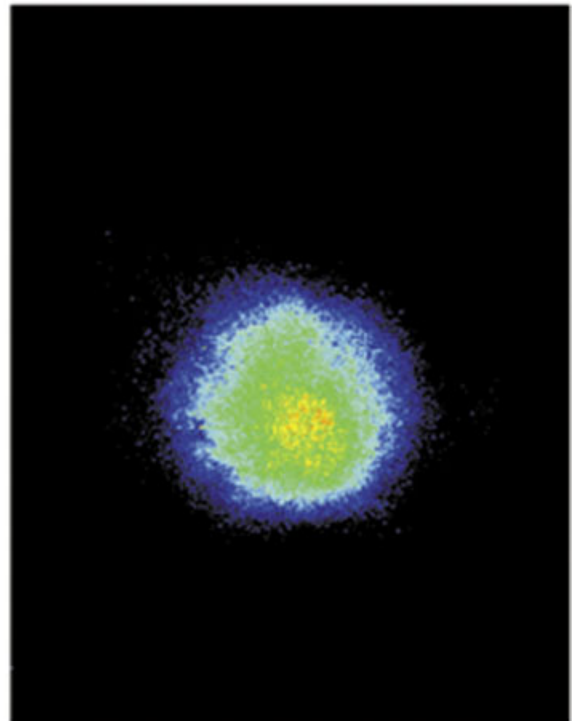
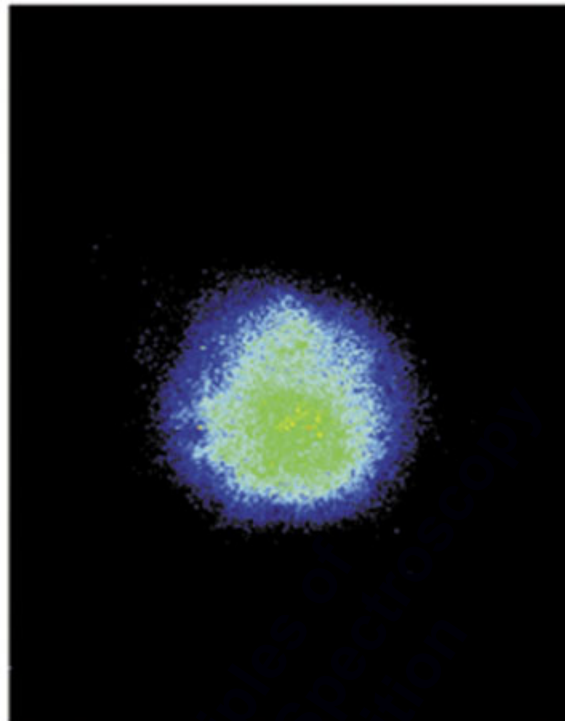
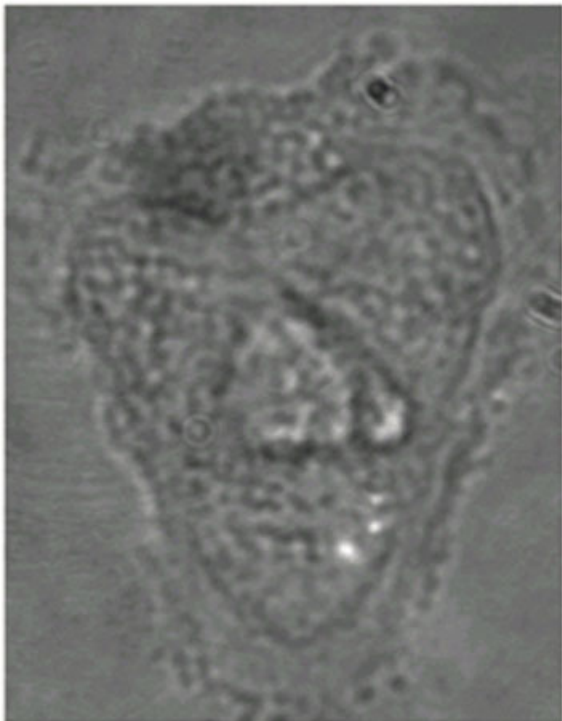


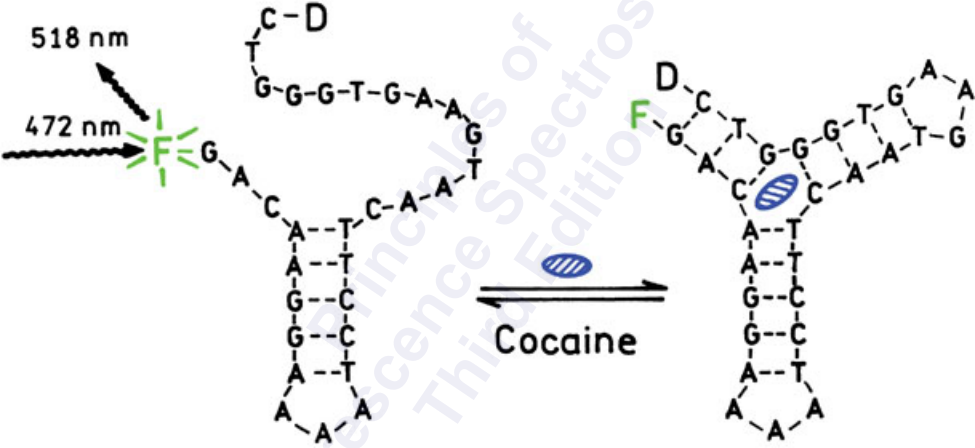


No target



With target







% Q

100

90

80

70

60

50

1

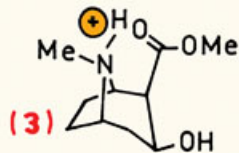
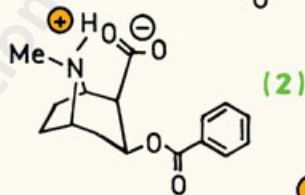
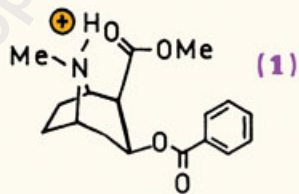
10

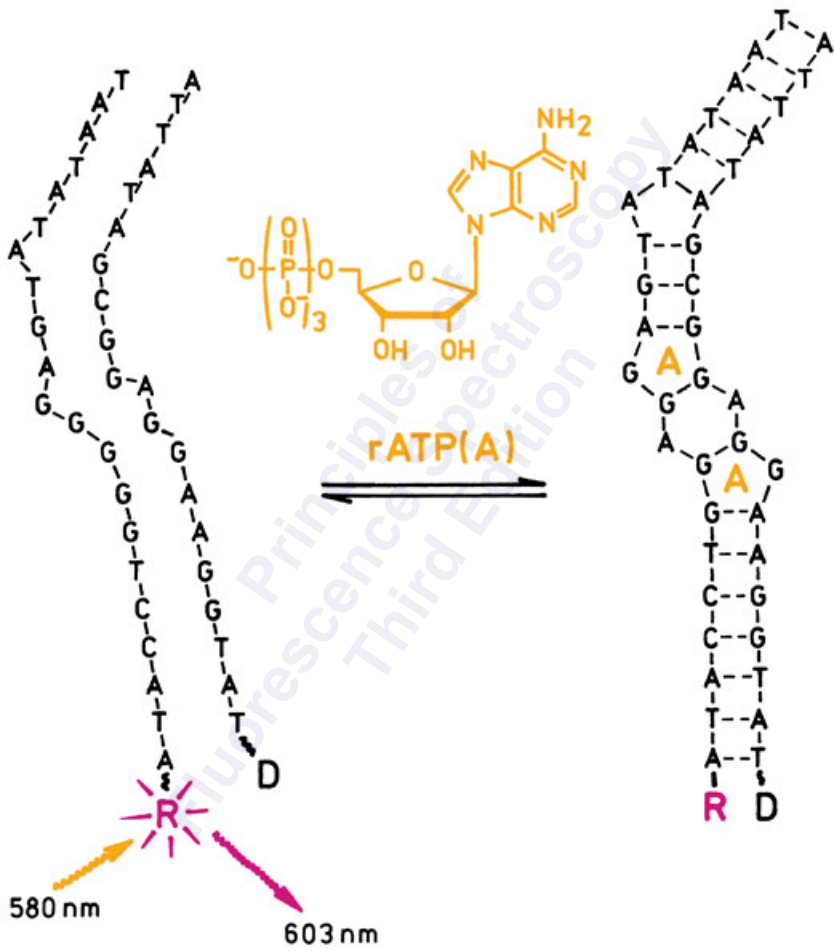
100

1000

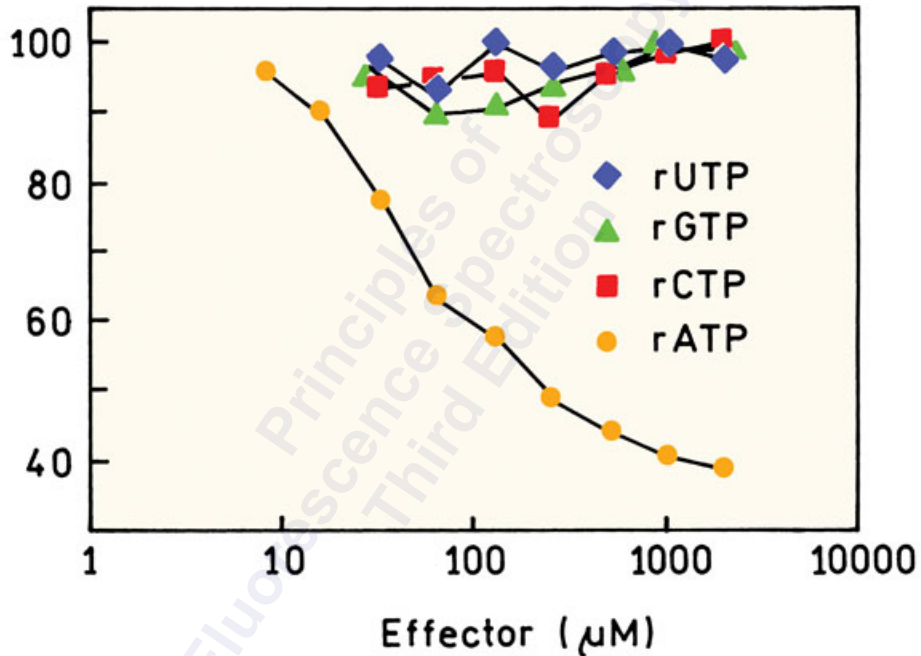
10000

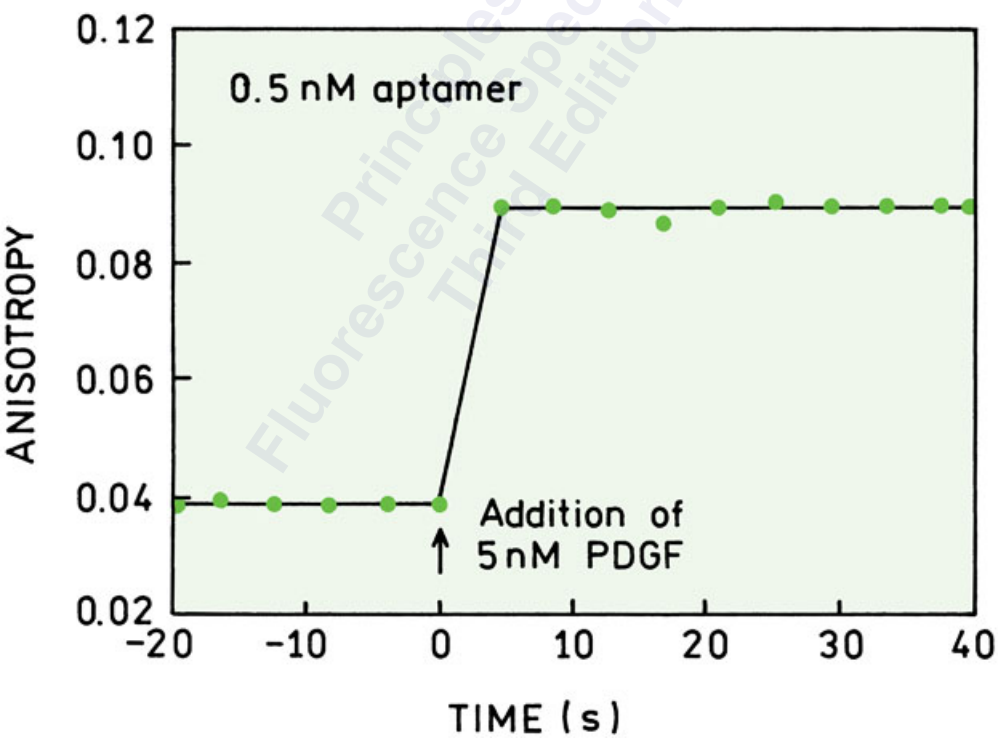
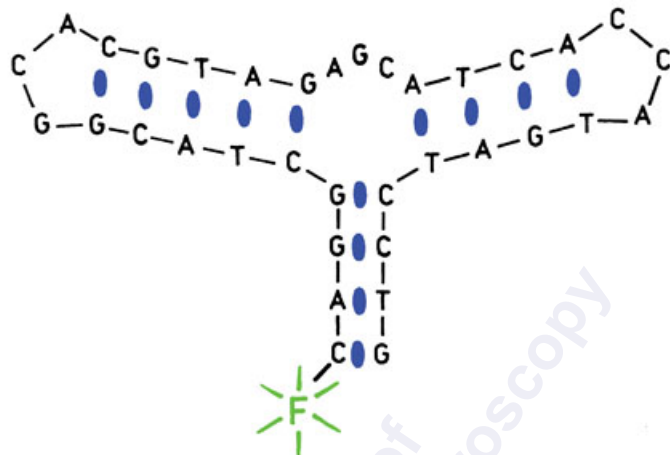
Concentration ( $\mu\text{M}$ )

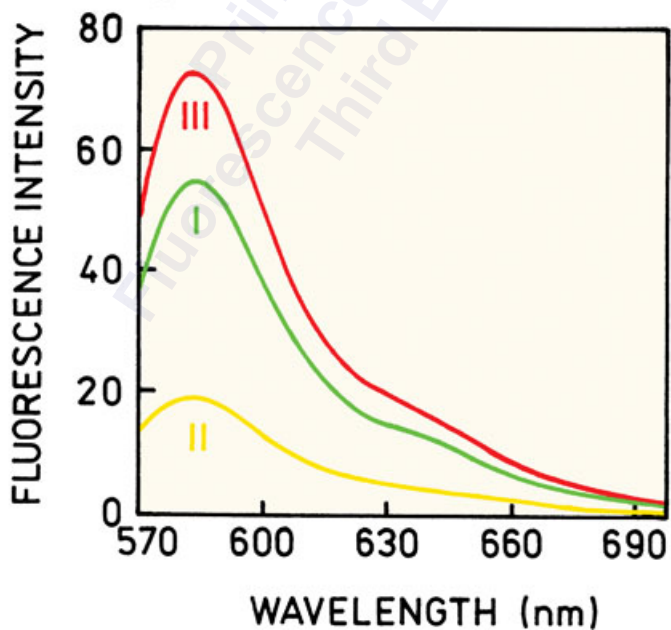
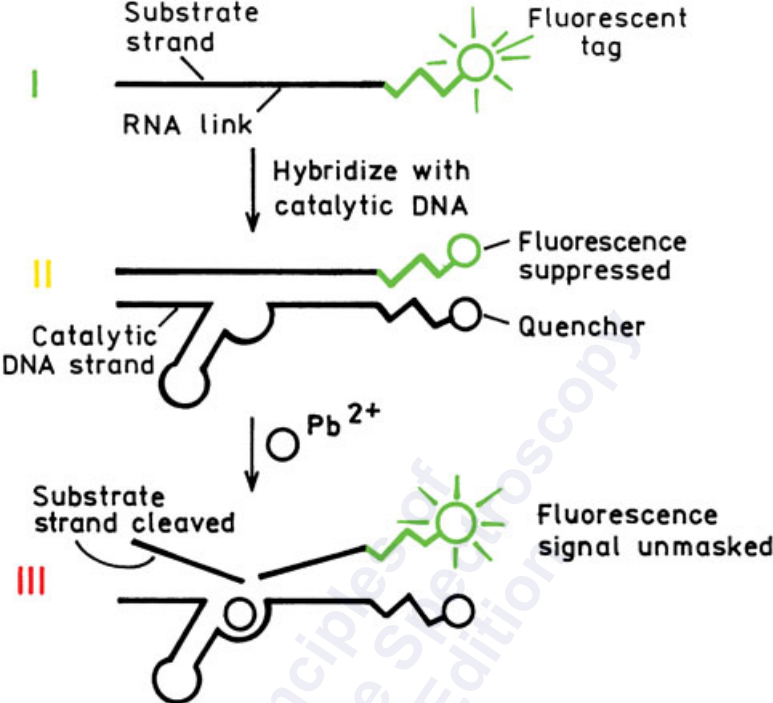


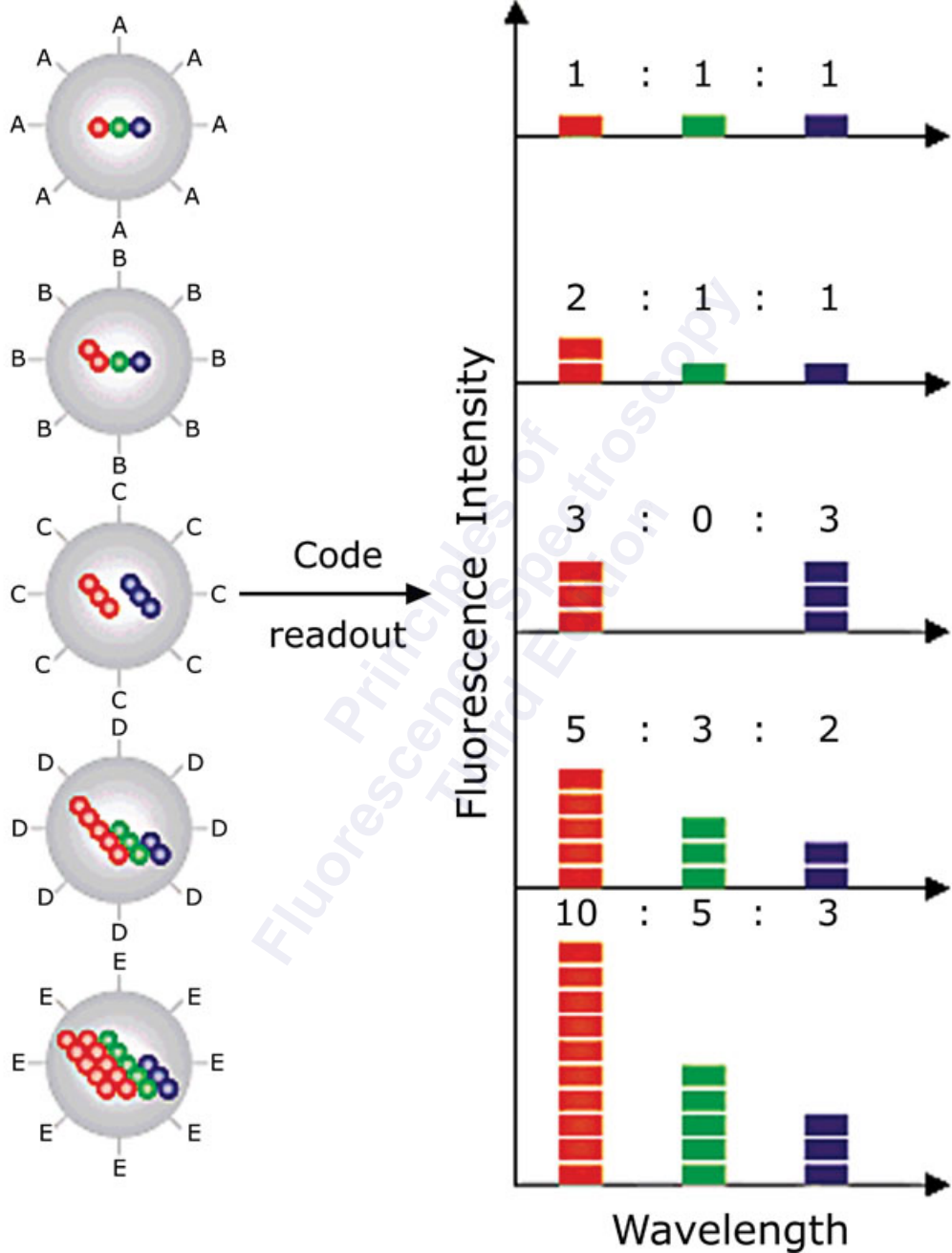


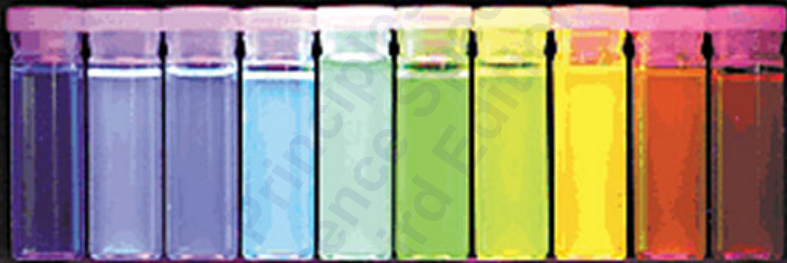
% Quench

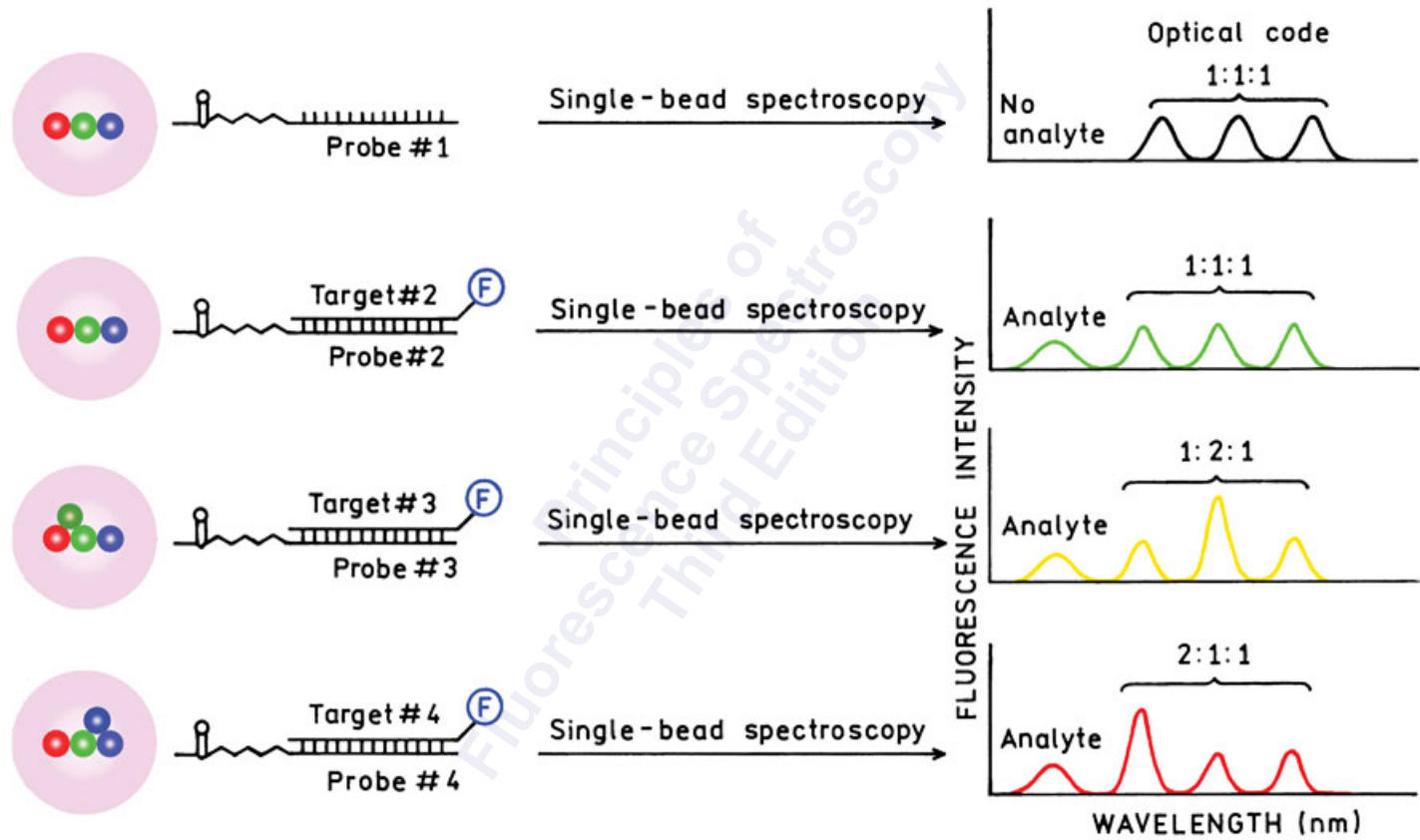






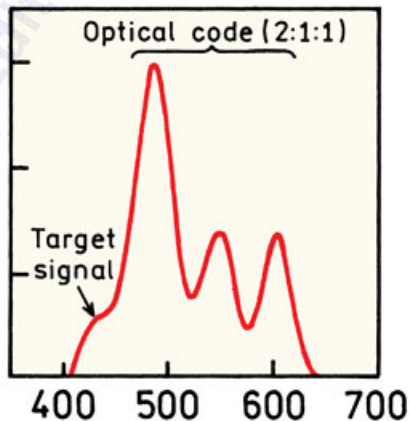
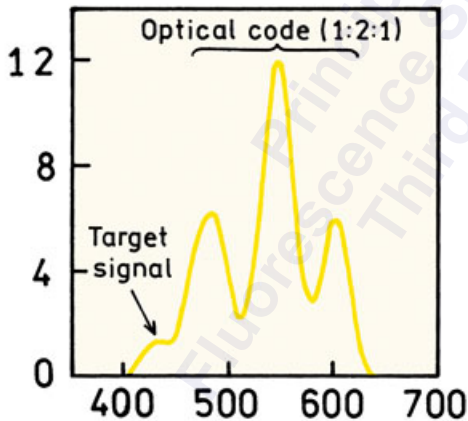
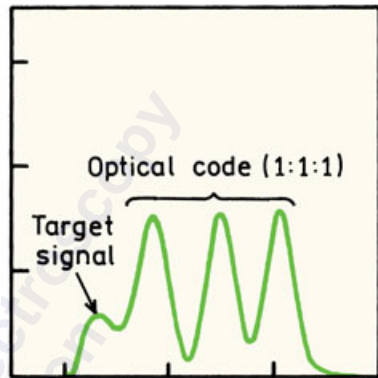
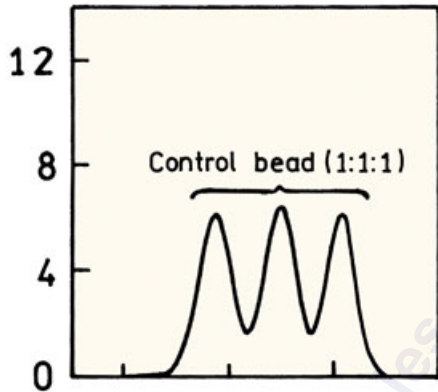




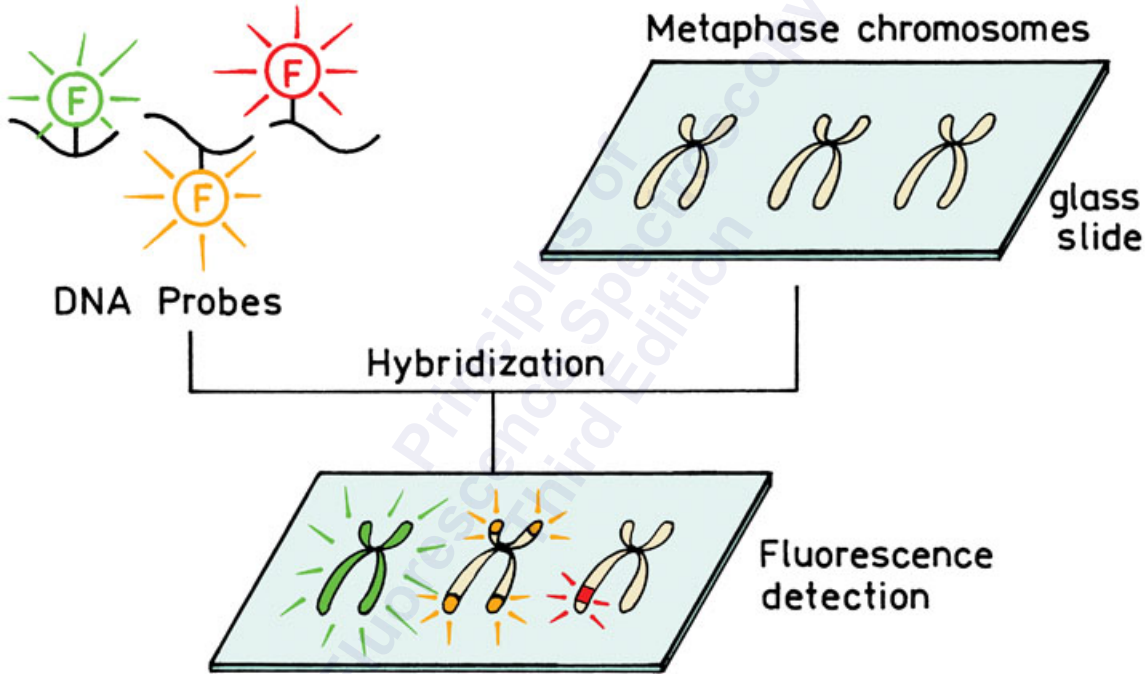


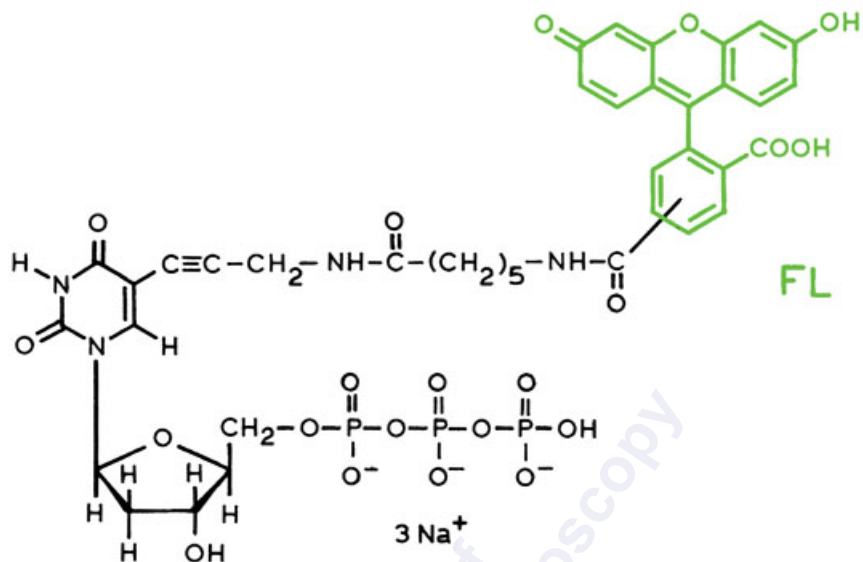


FLUORESCENCE INTENSITY

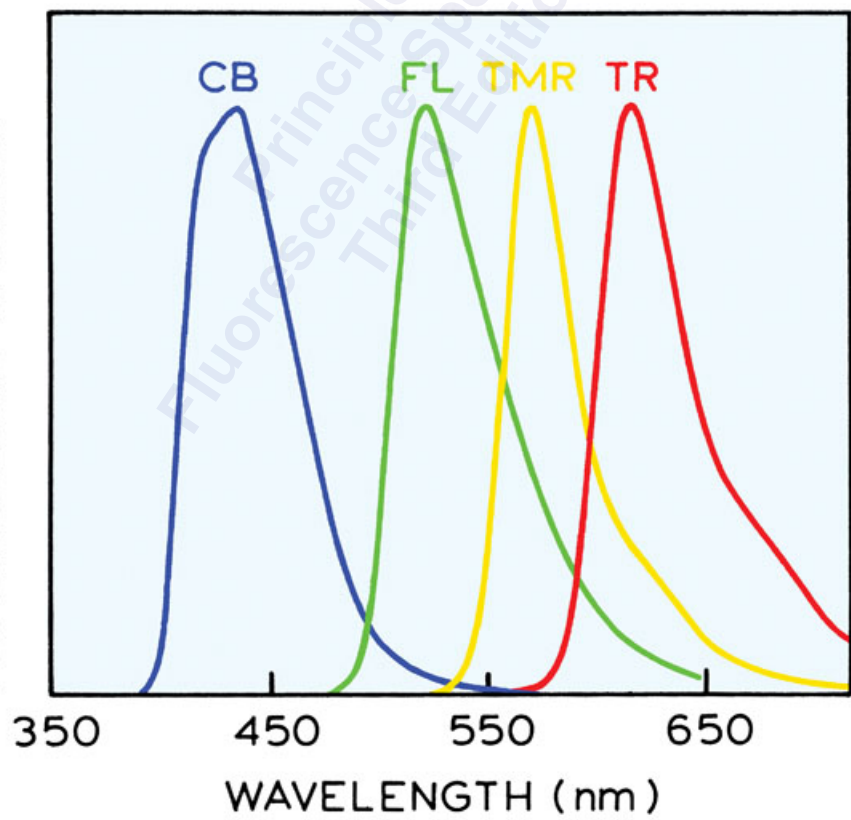


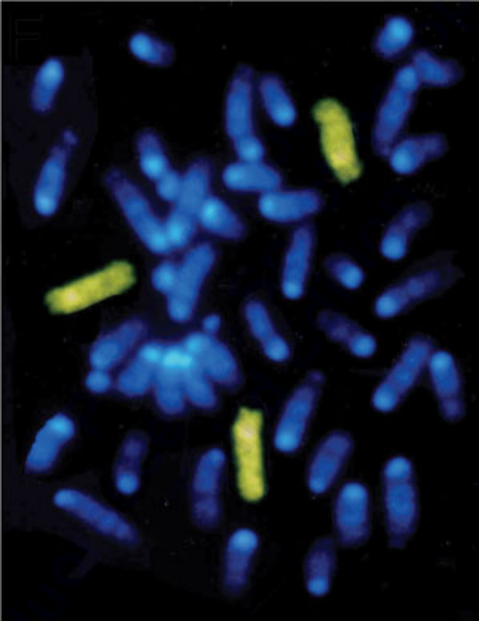
WAVELENGTH (nm)

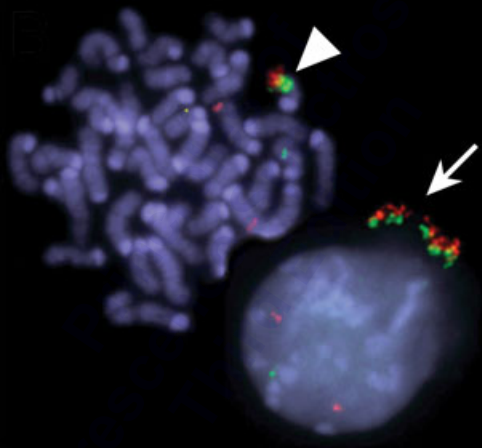


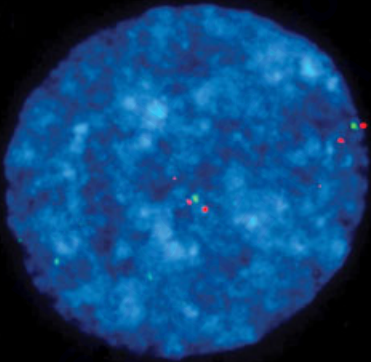


FLUORESCENCE EMISSION

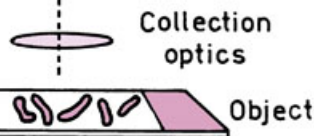
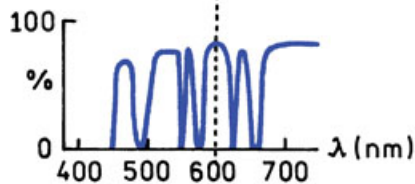
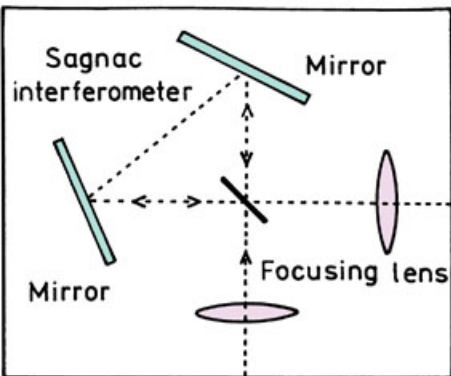




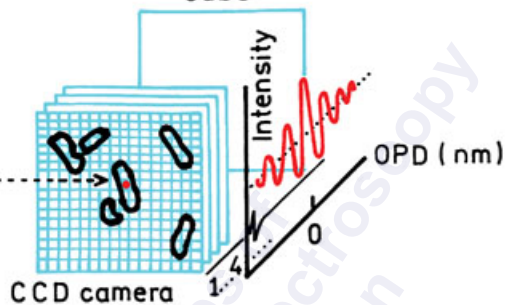




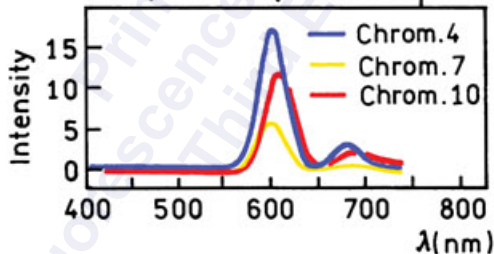
## Optical head



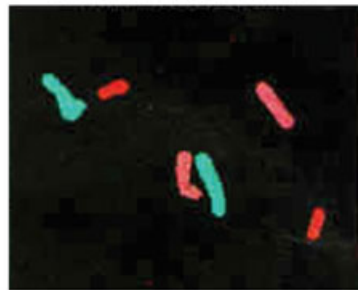
## Interferogram cube



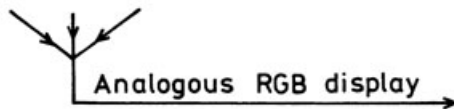
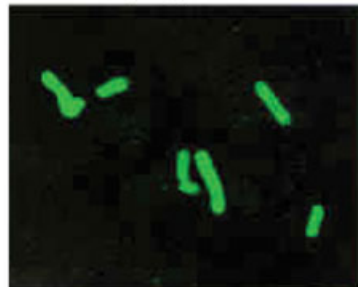
Fourier transformation  
Emission spectra  
Mathematical pixel classification



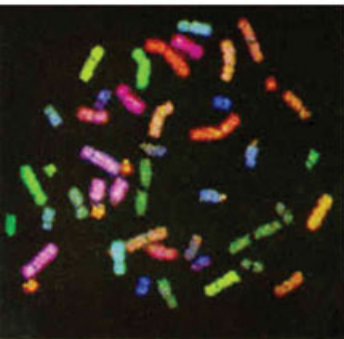
## Classification colors



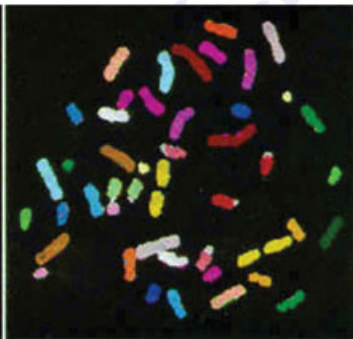
## Display colors



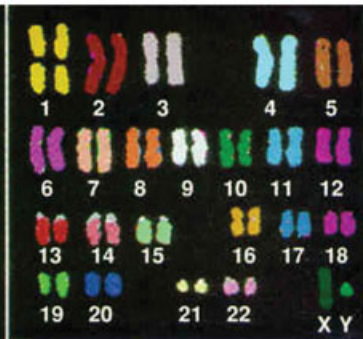
Display Colors



Clasifcation Colors

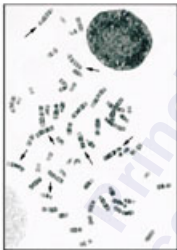


Sorted Chromosomes





Inverse DAPI

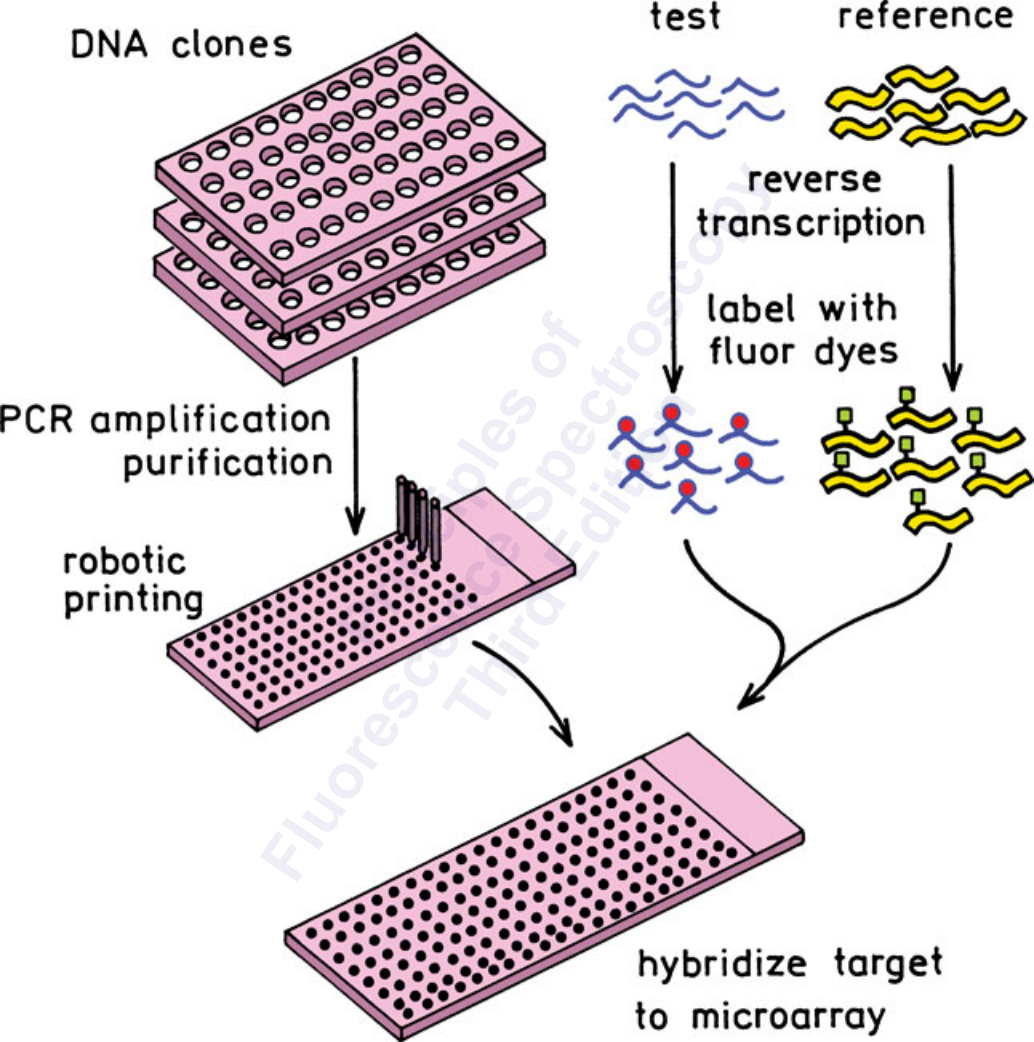


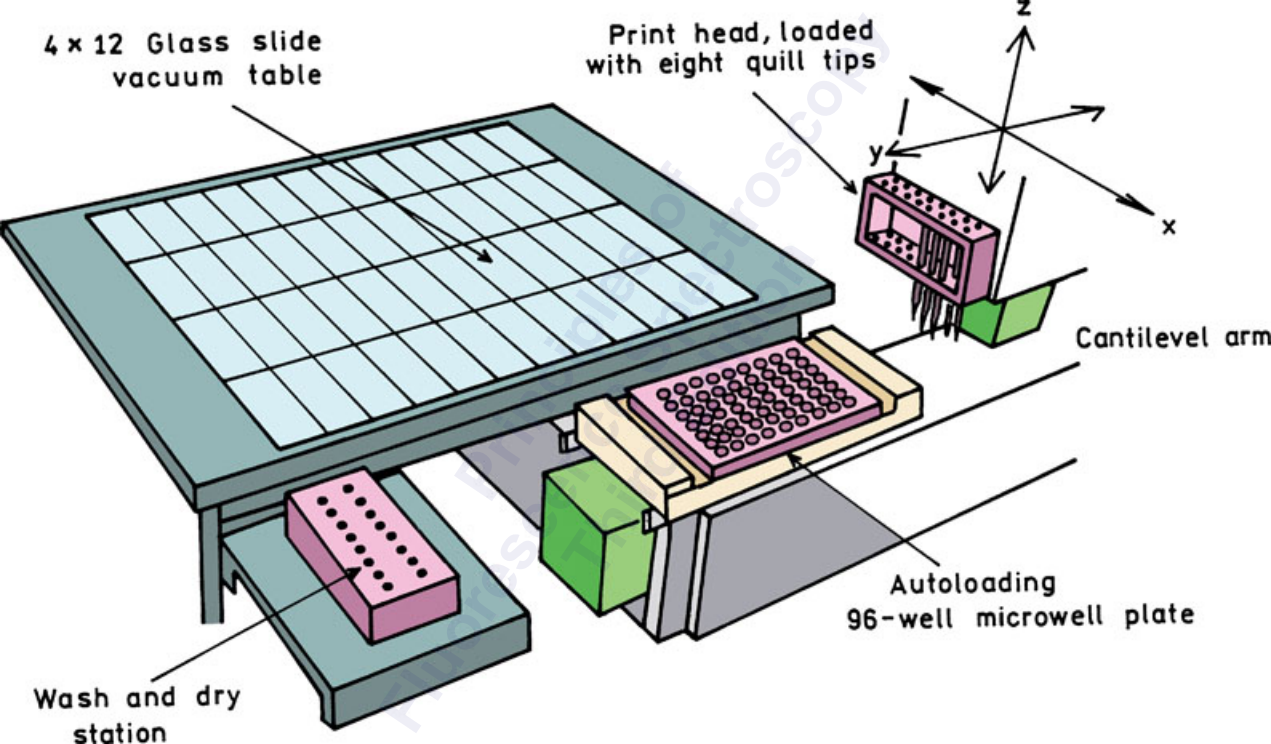
RGB



SKY







Control

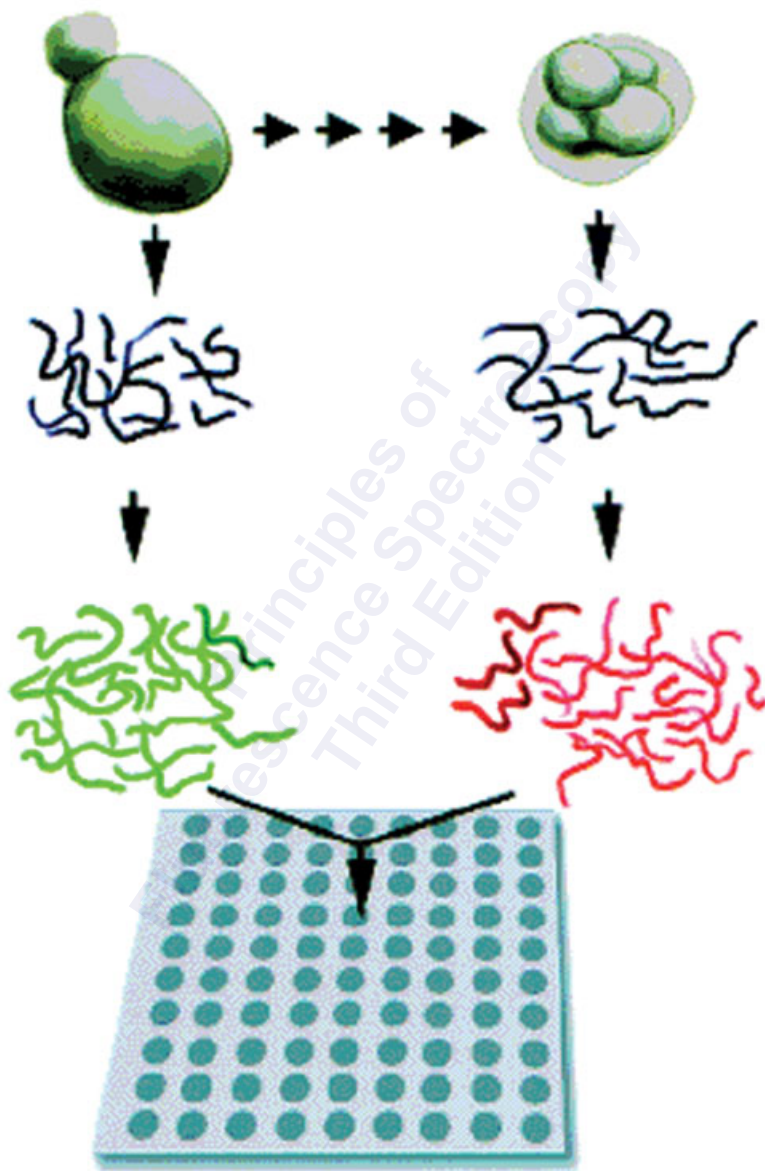
Sample

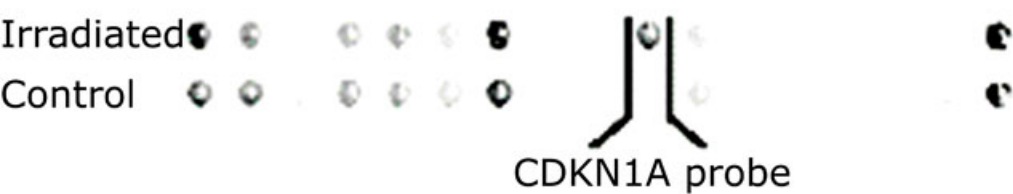
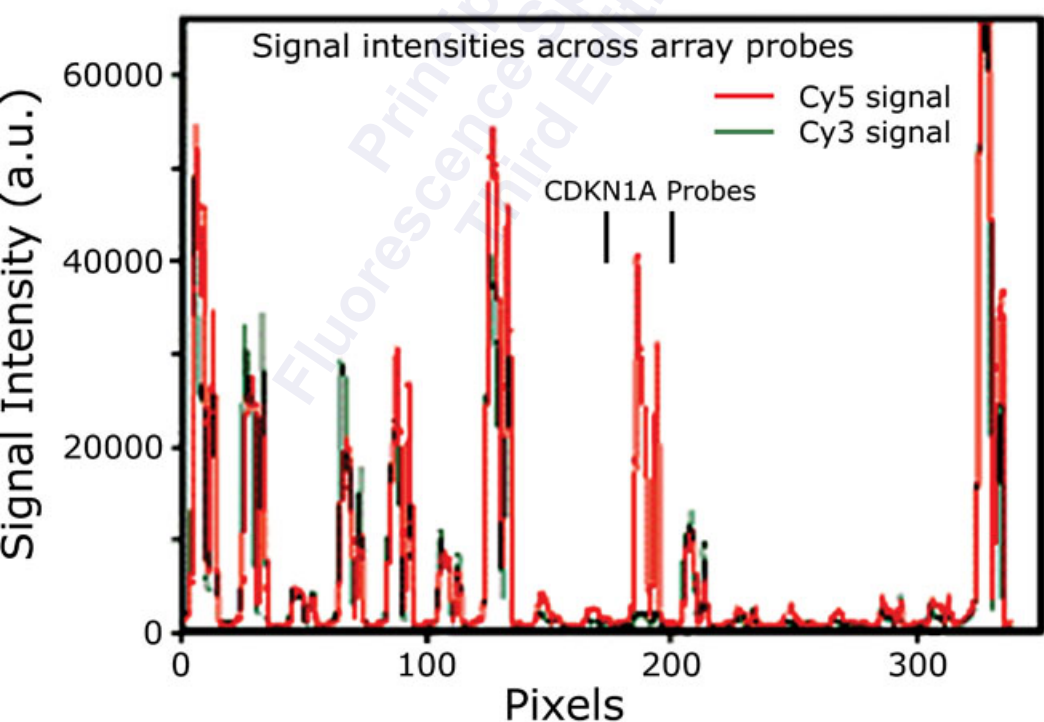
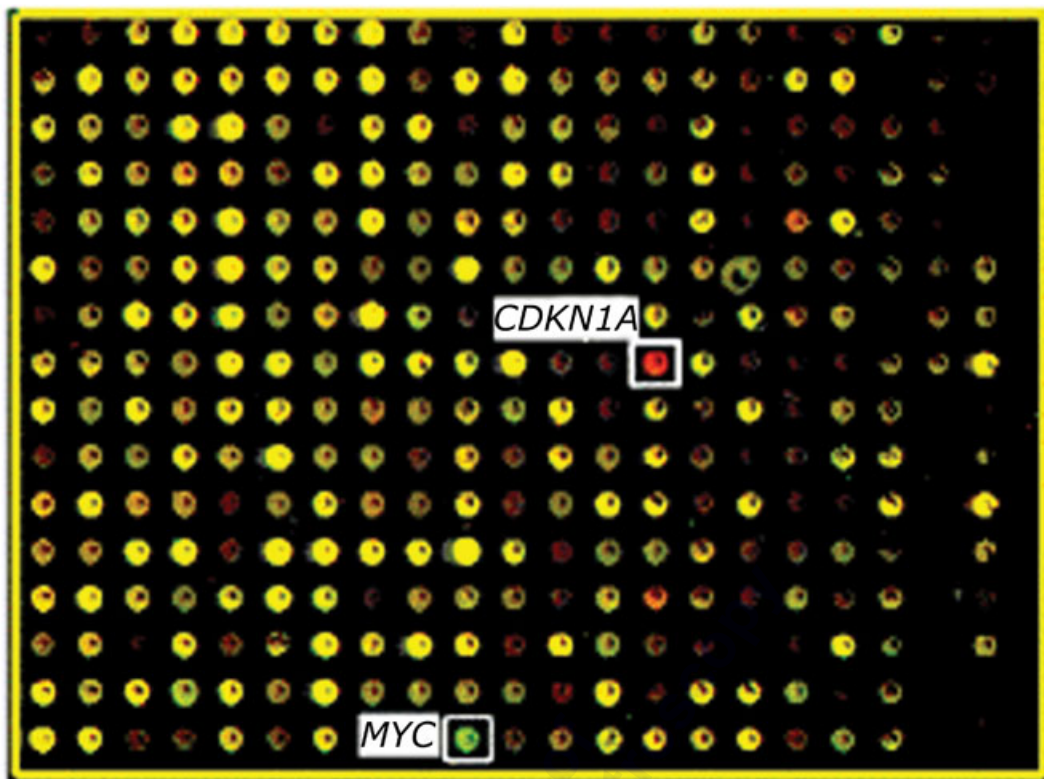
mRNA

Cy3  
cDNA

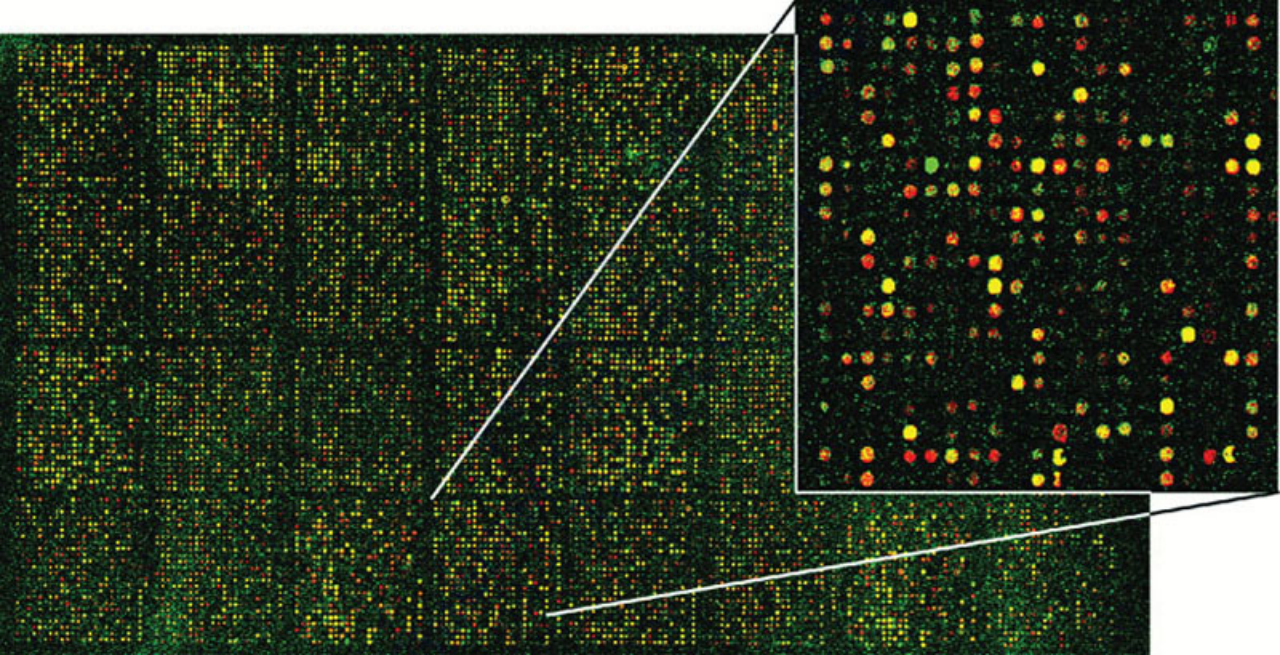
Cy5  
cDNA

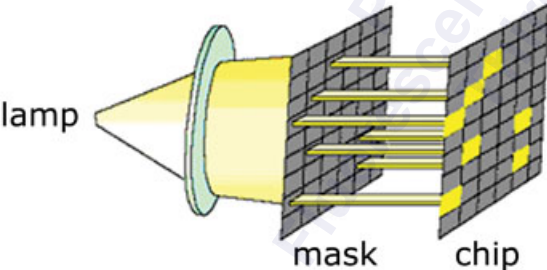
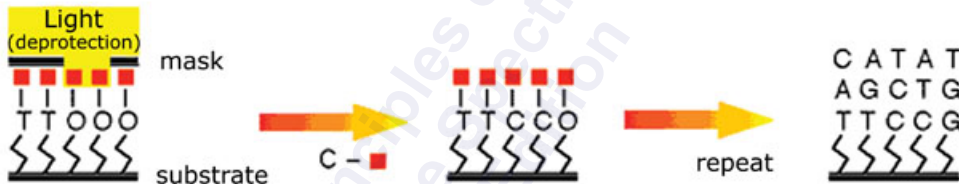
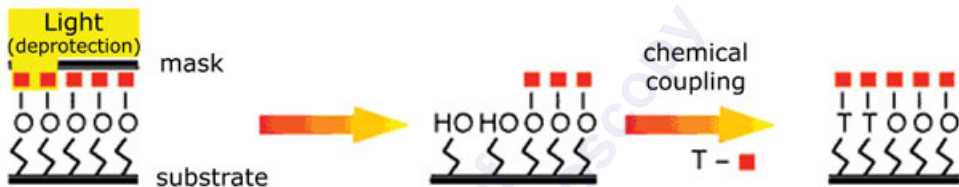
DNA microarray

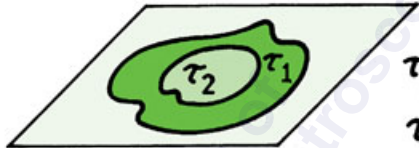






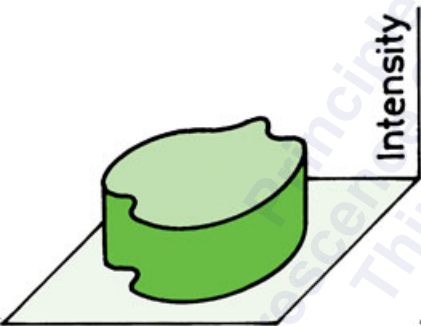




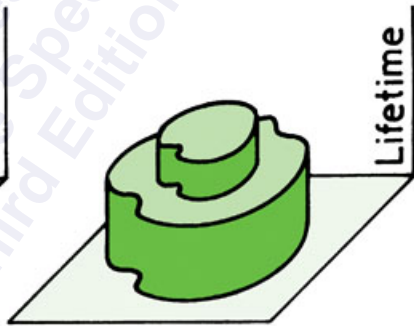


$$\tau_1 = f[\text{Ca}^{2+}]_1$$

$$\tau_2 = f[\text{Ca}^{2+}]_2$$

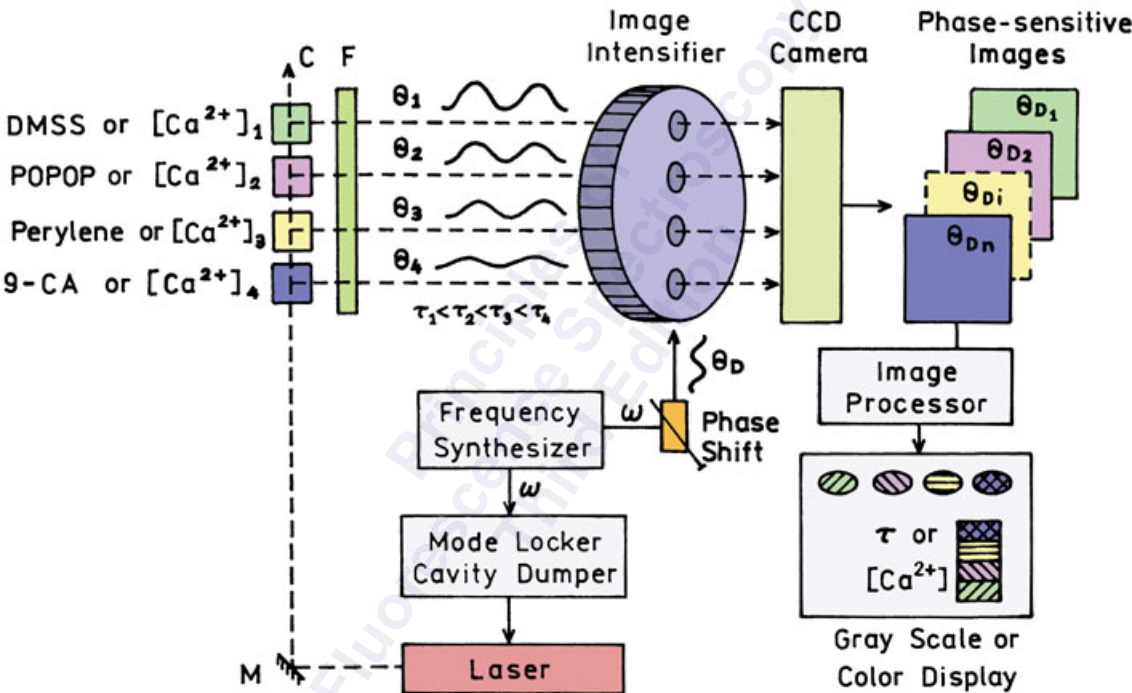


Intensity Image



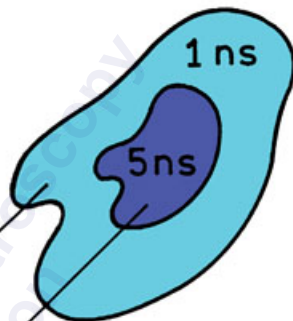
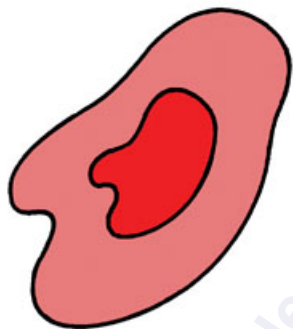
Lifetime Image



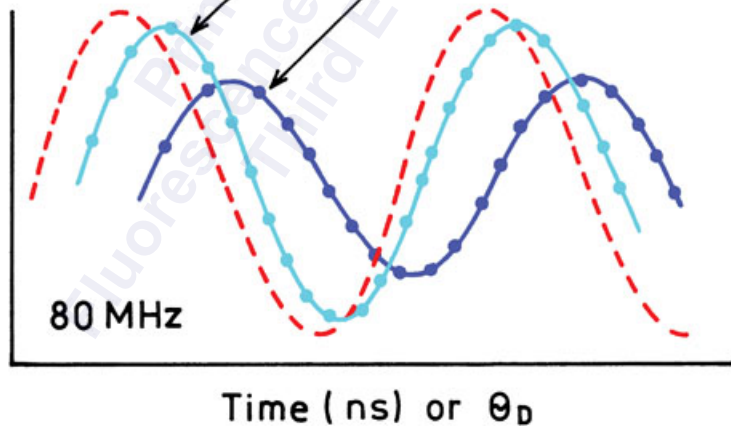


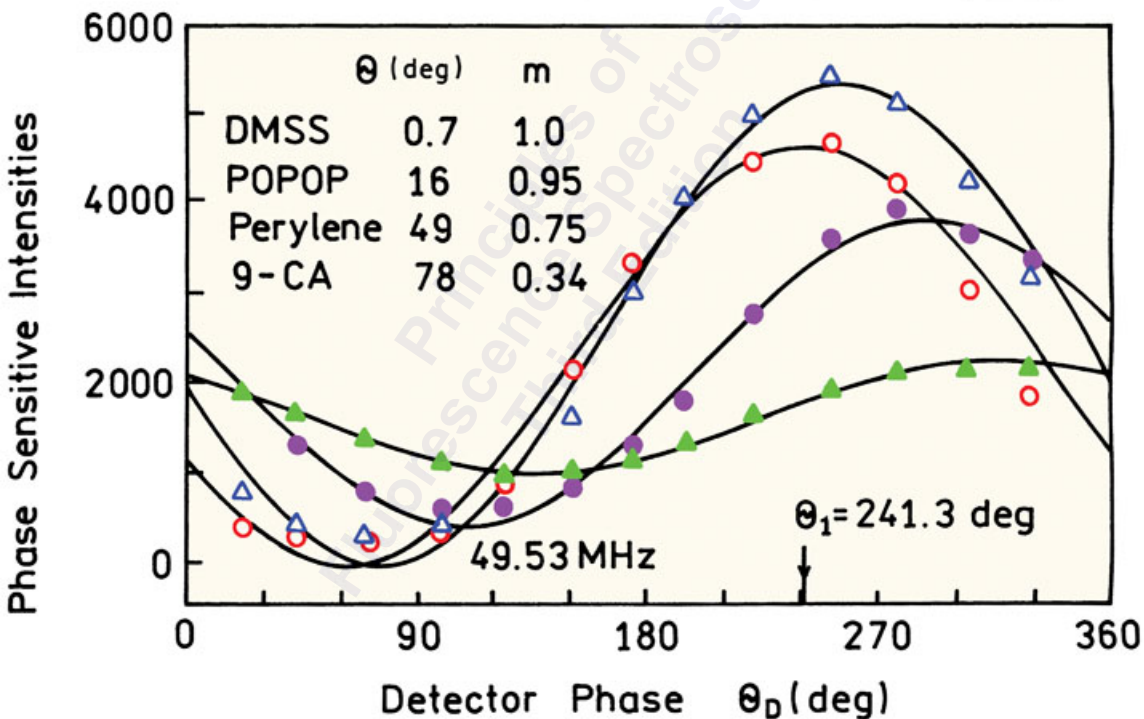
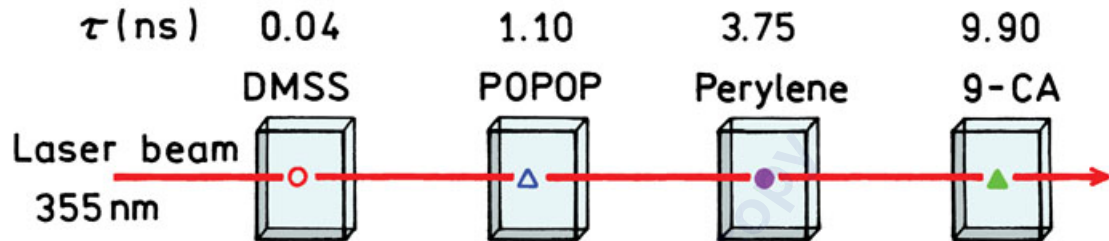
Intensity

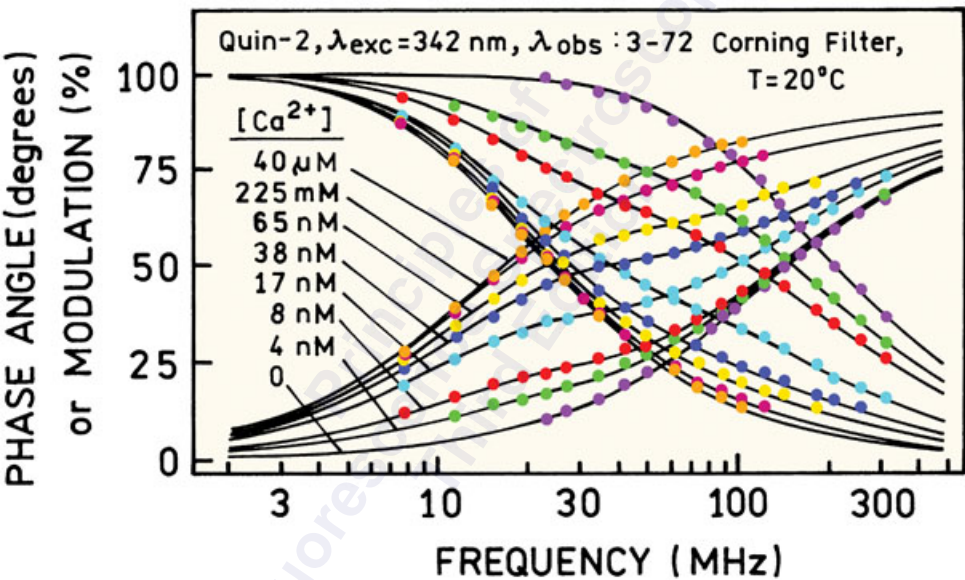
Lifetime



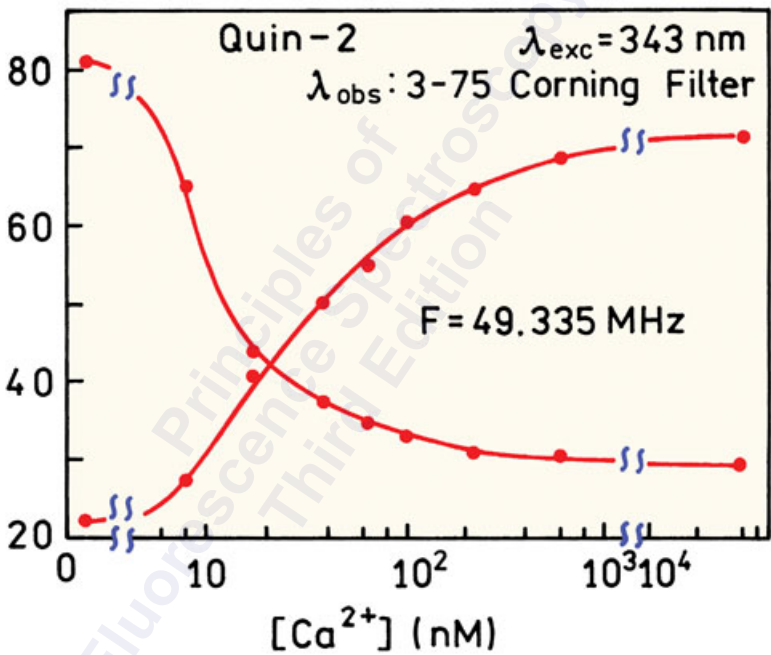
Phase Sensitive  
Intensity

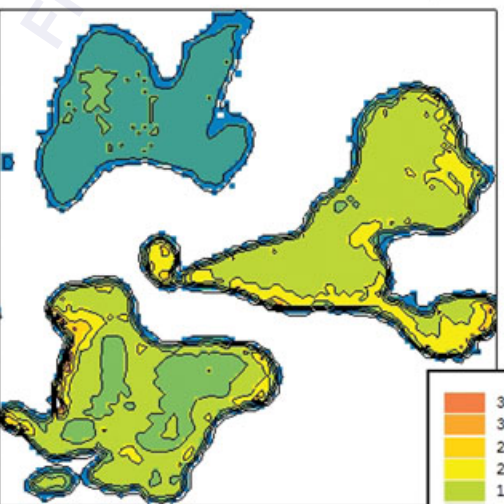
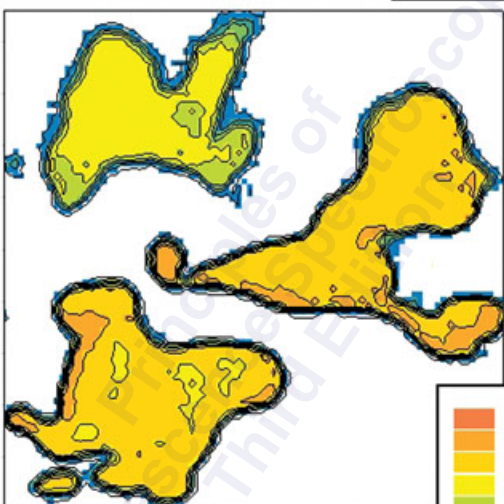
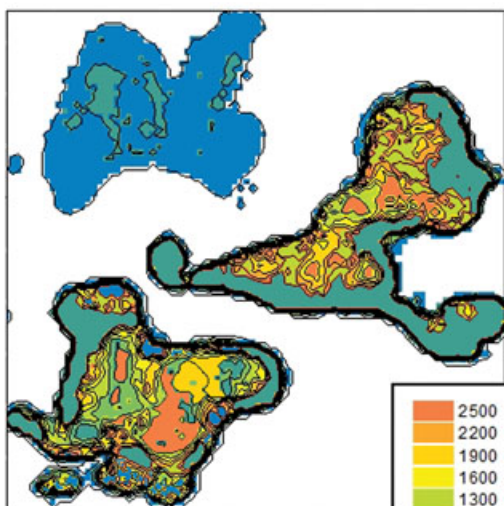






PHASE ANGLE (degrees)  
or MODULATION (%)



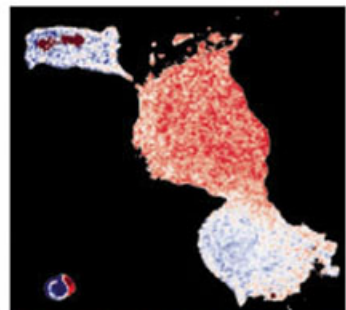
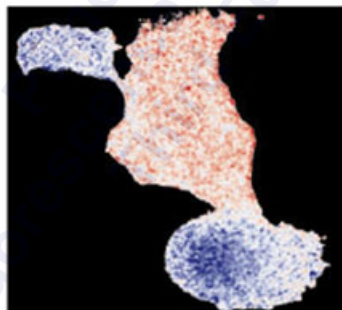
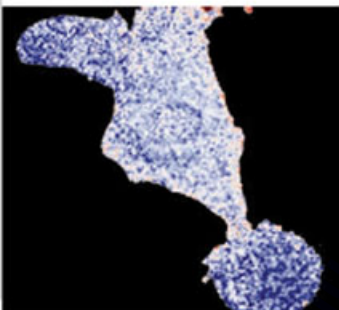
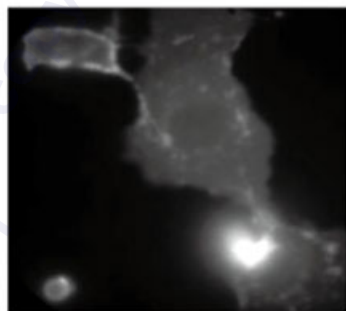
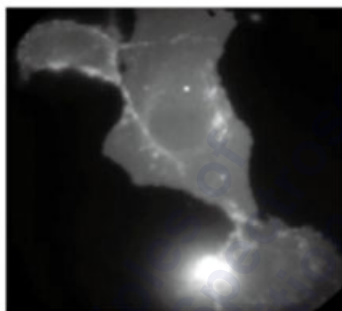
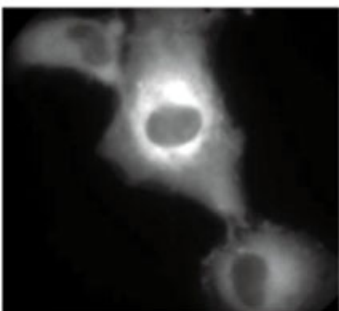


PMA (min)

0

20

40

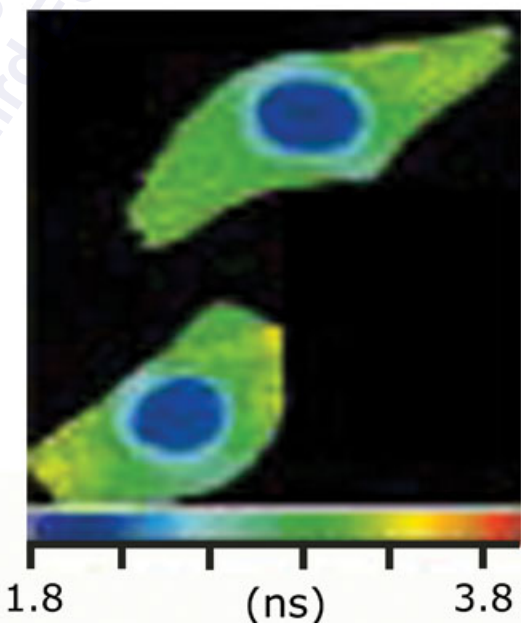
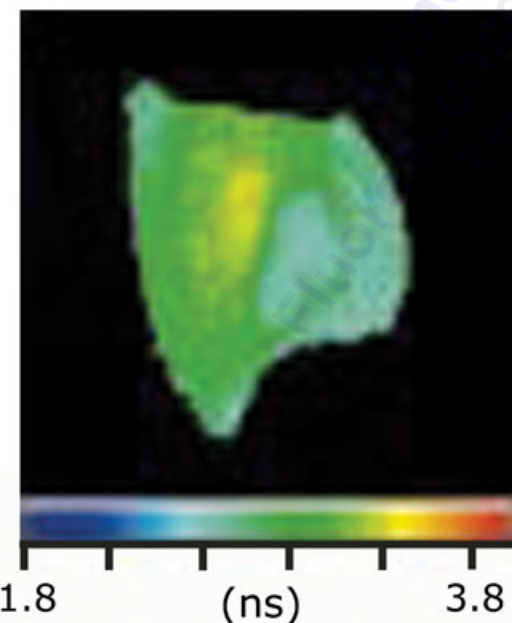
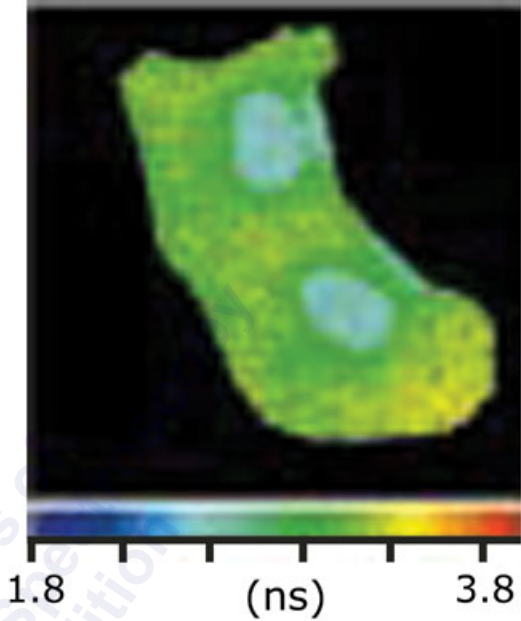
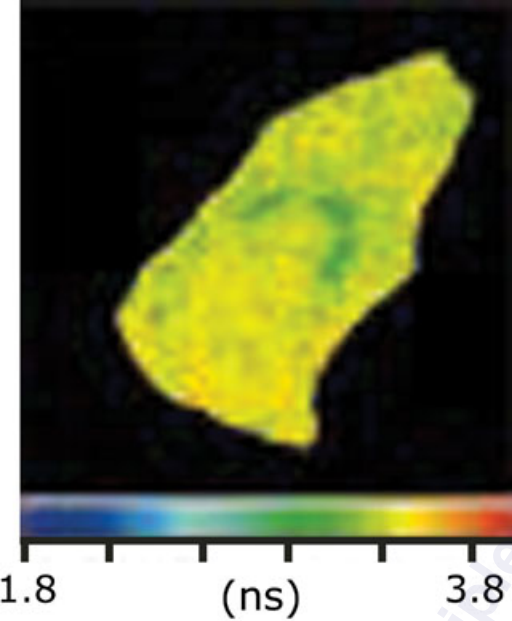


1.5

$\tau(\text{ns})$

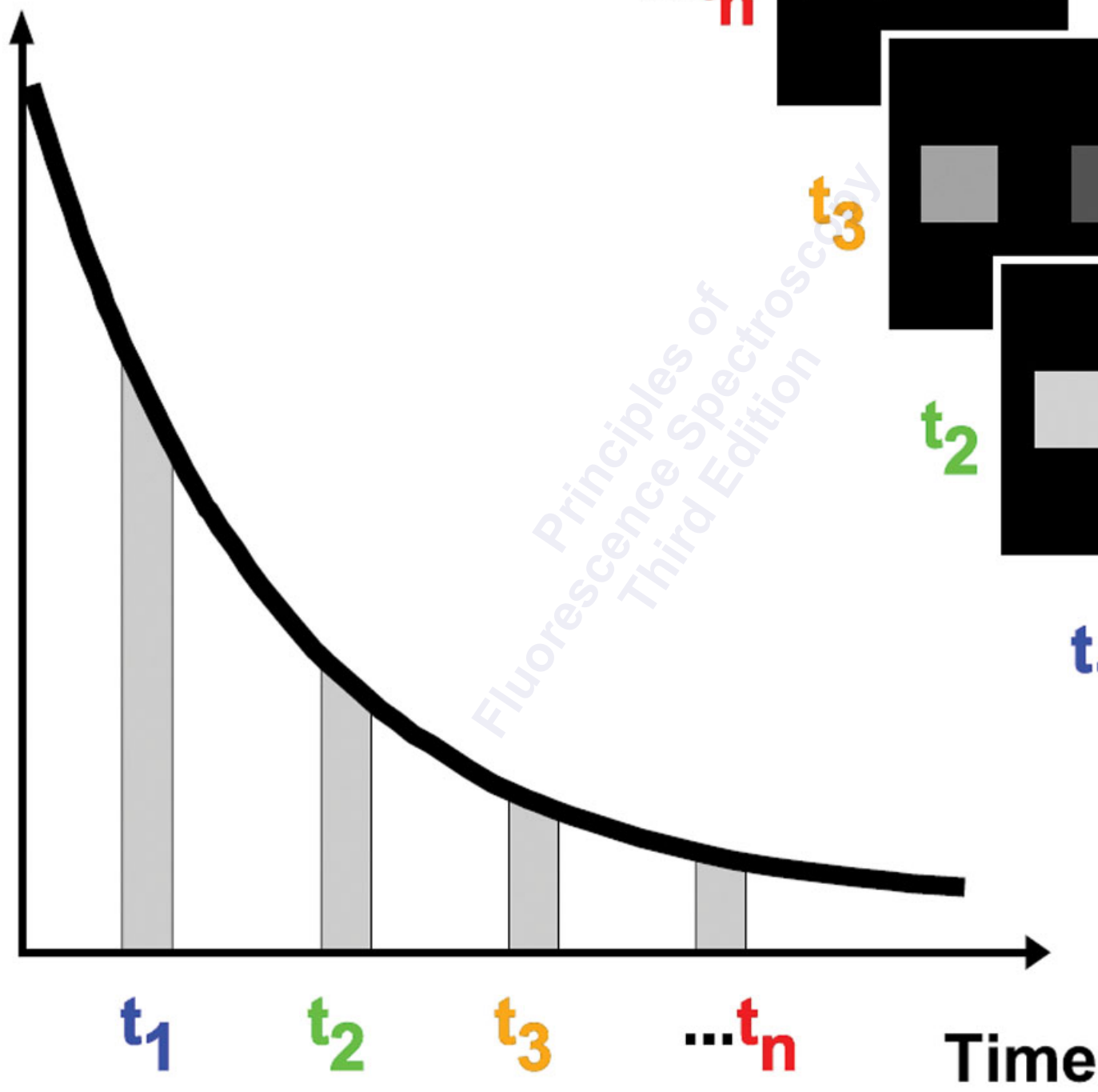
2.9

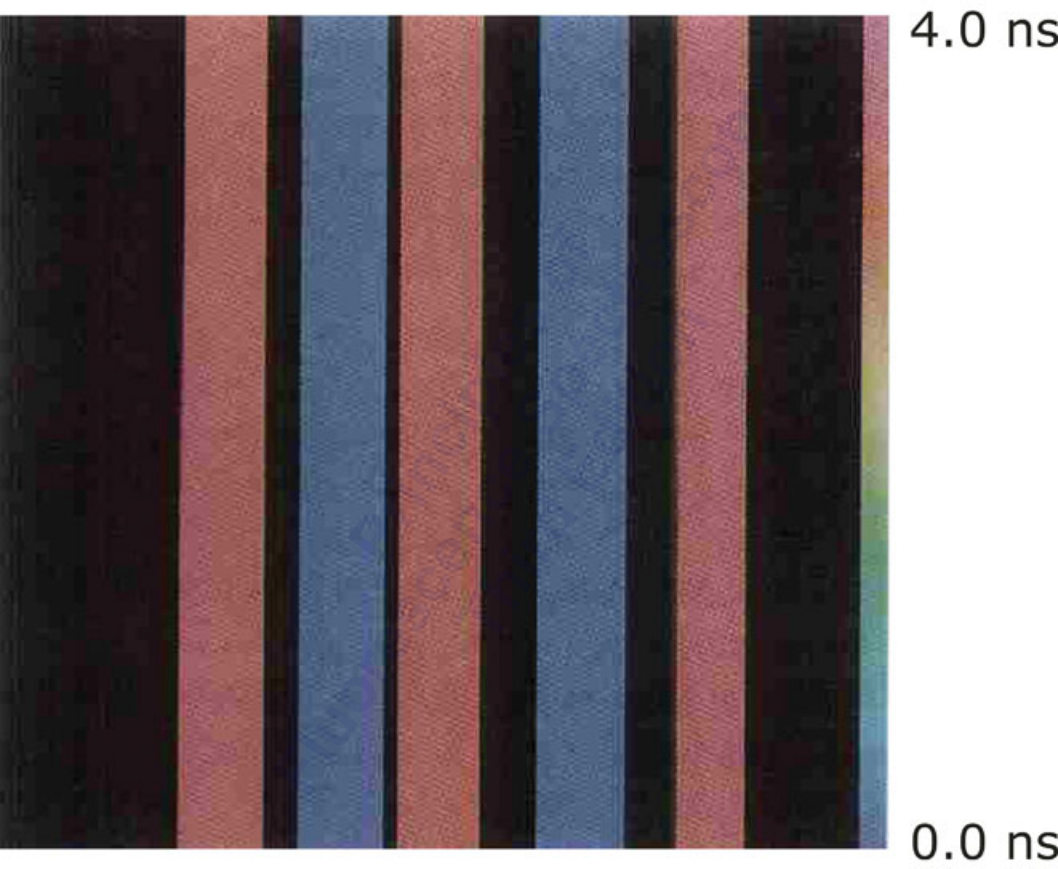






Intensity





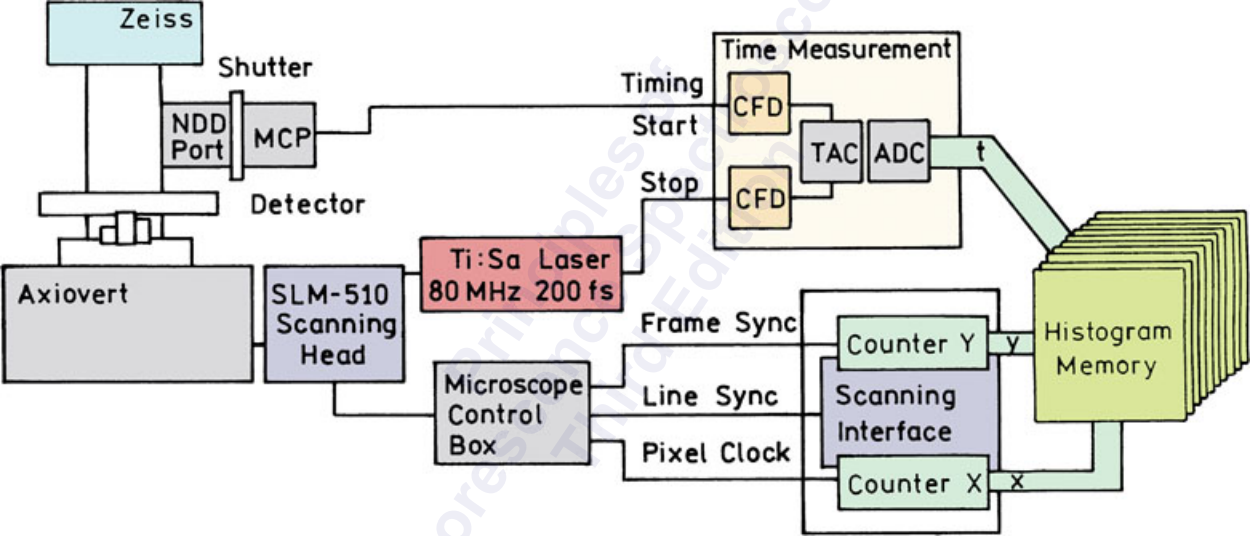
2.55 ns

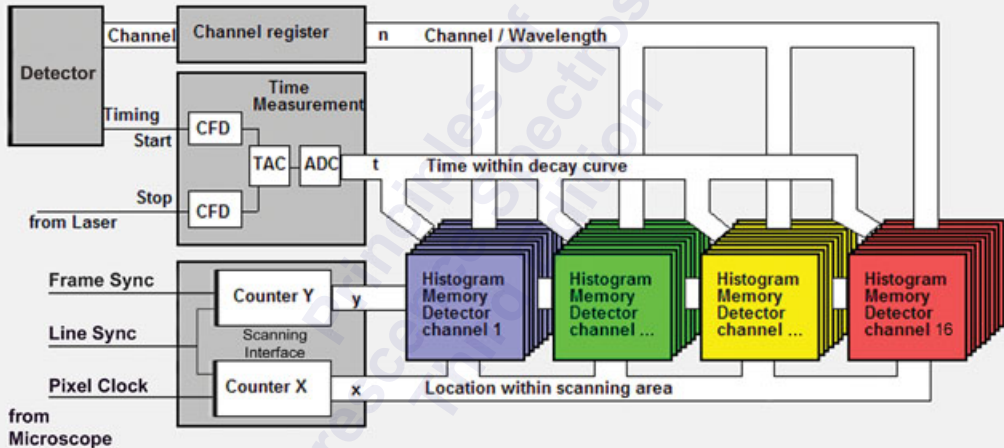
1.28 ns

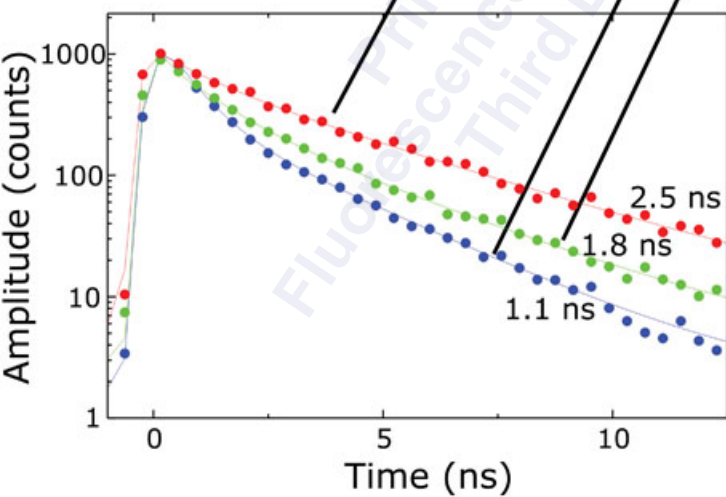
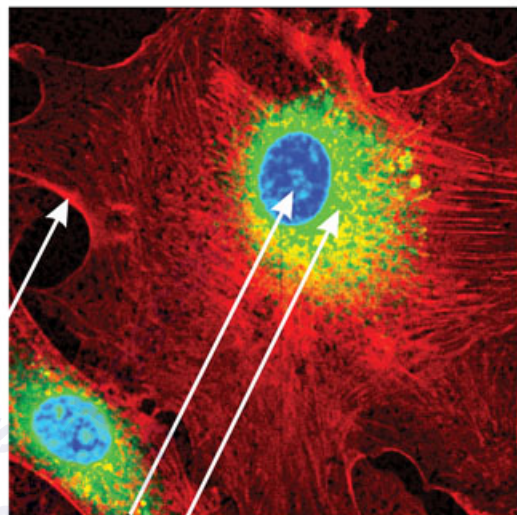
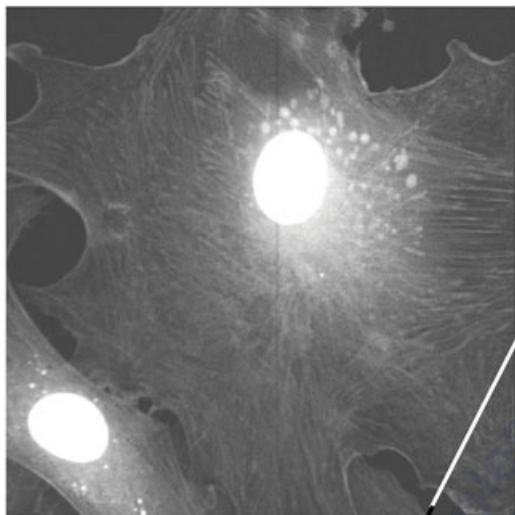
0 ns



10  $\mu\text{m}$







Lifetime (ps)

2500



2000

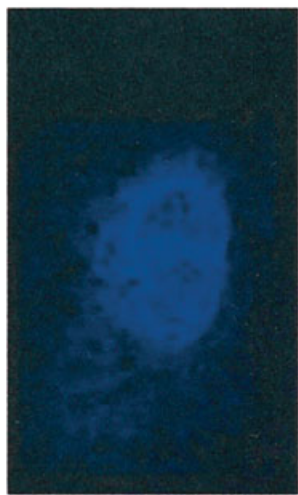


1500

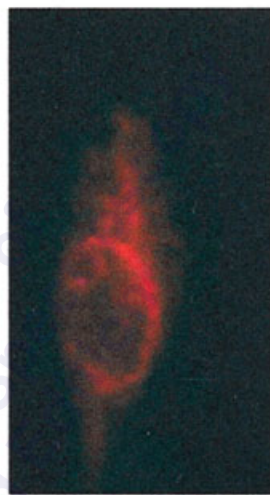
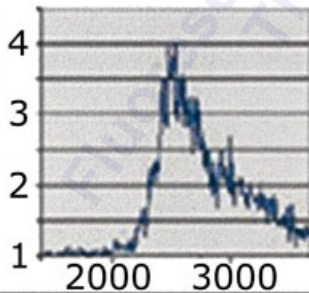


1000

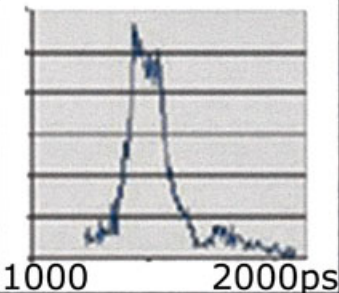


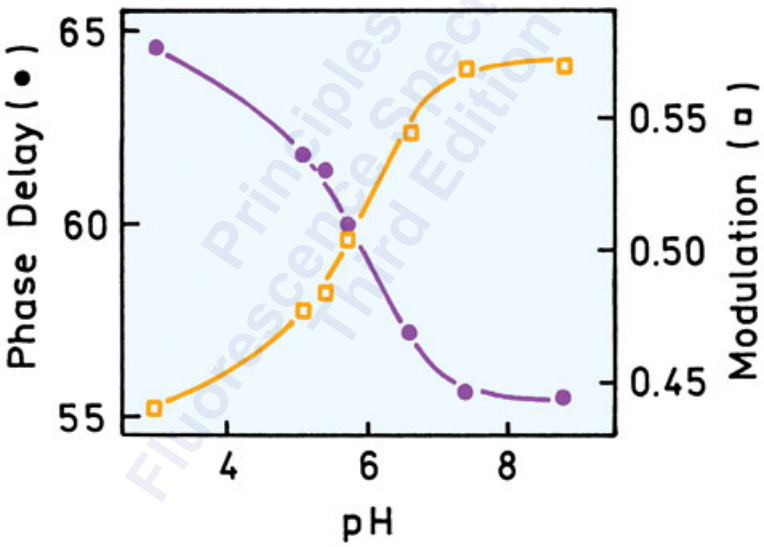
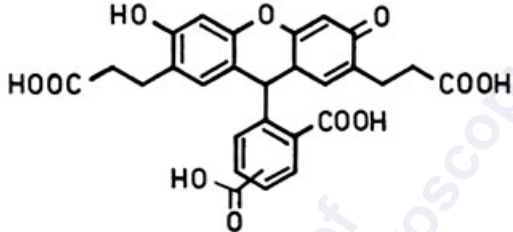


1000 2500



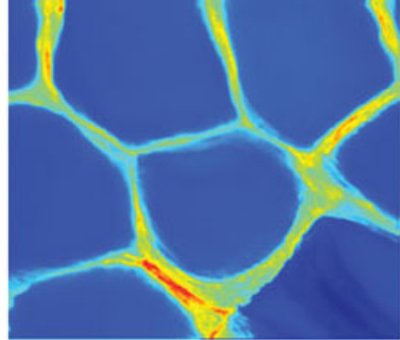
1000 2500







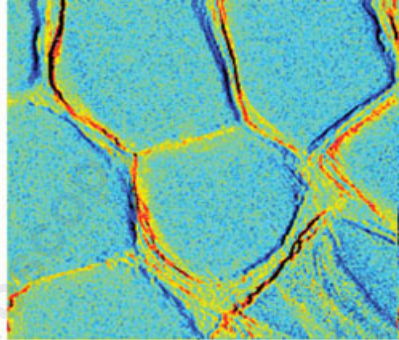
Intensity



100

1000

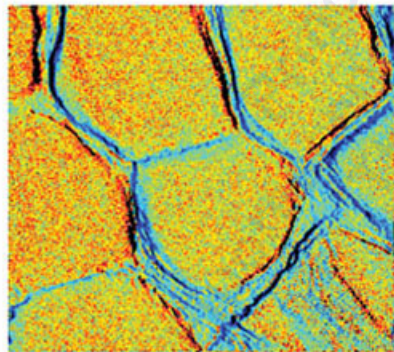
Modulation  
uncorrected for  $\eta$



0.2

0.7

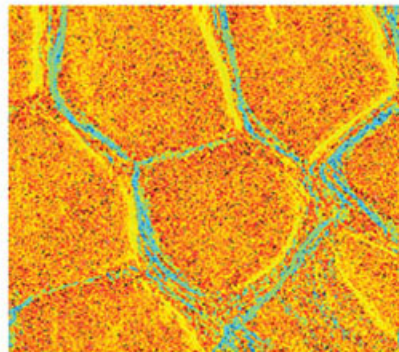
$\tau$  (ns)  
uncorrected for  $\eta$



2

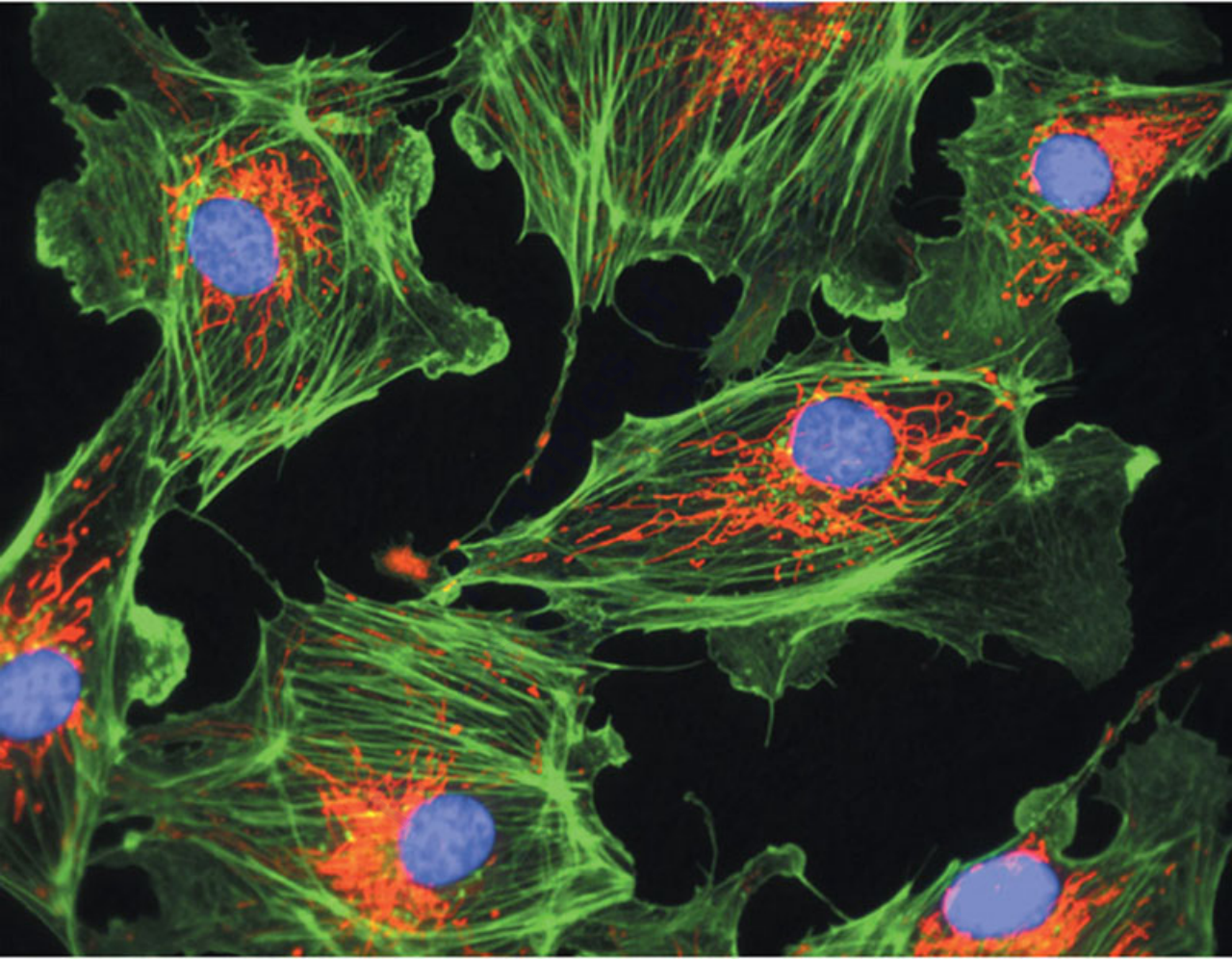
5

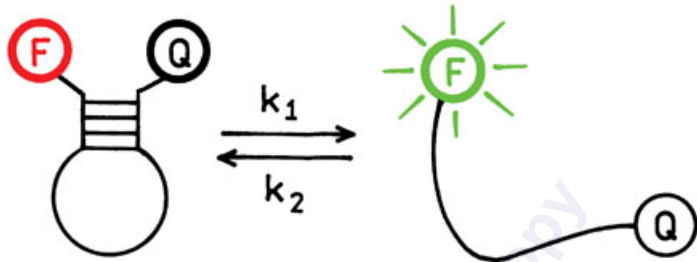
pH  
corrected for  $\eta$



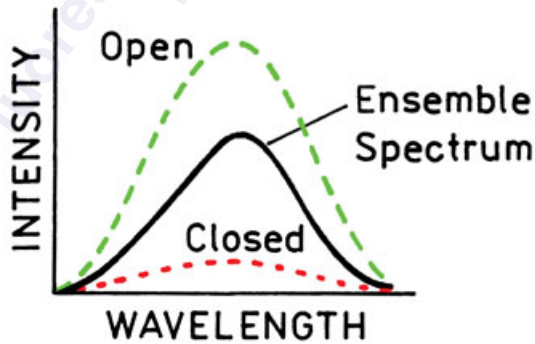
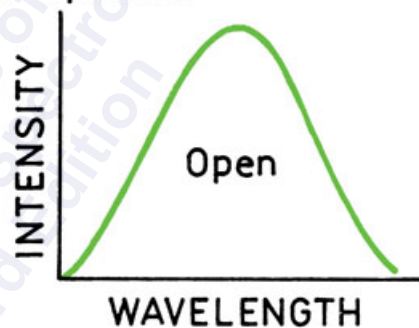
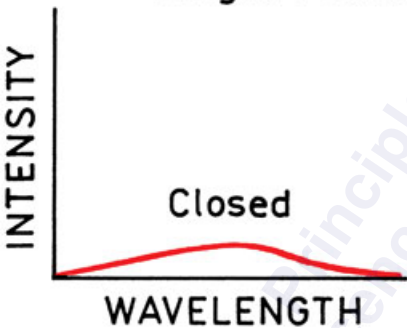
4.0

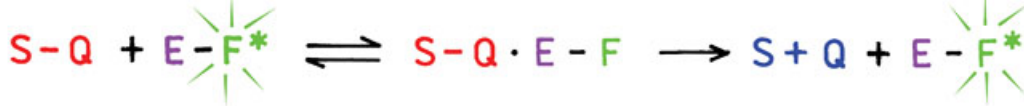
8.0





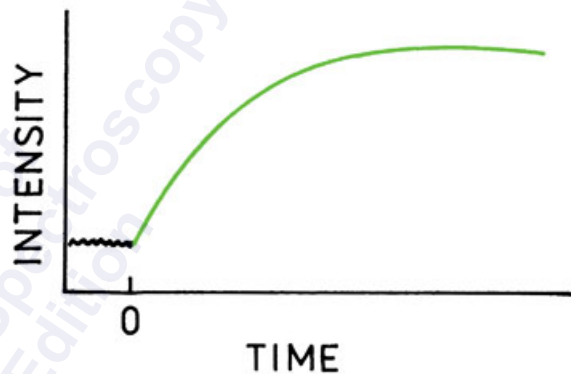
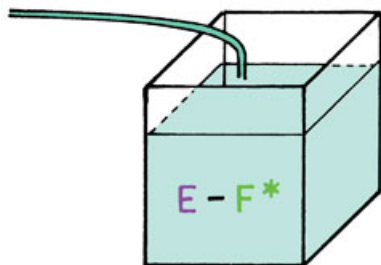
### Single Molecule Spectra



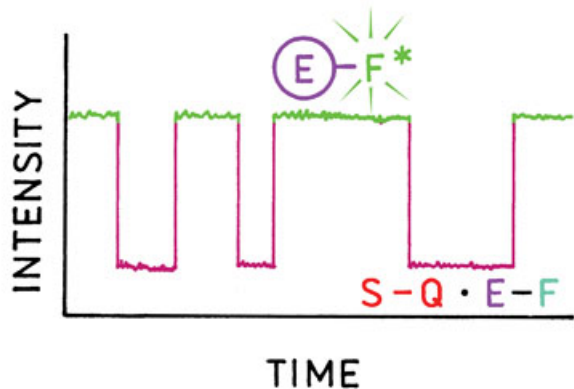
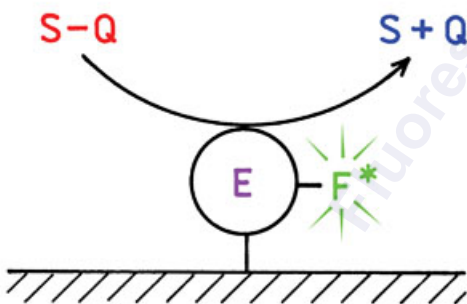


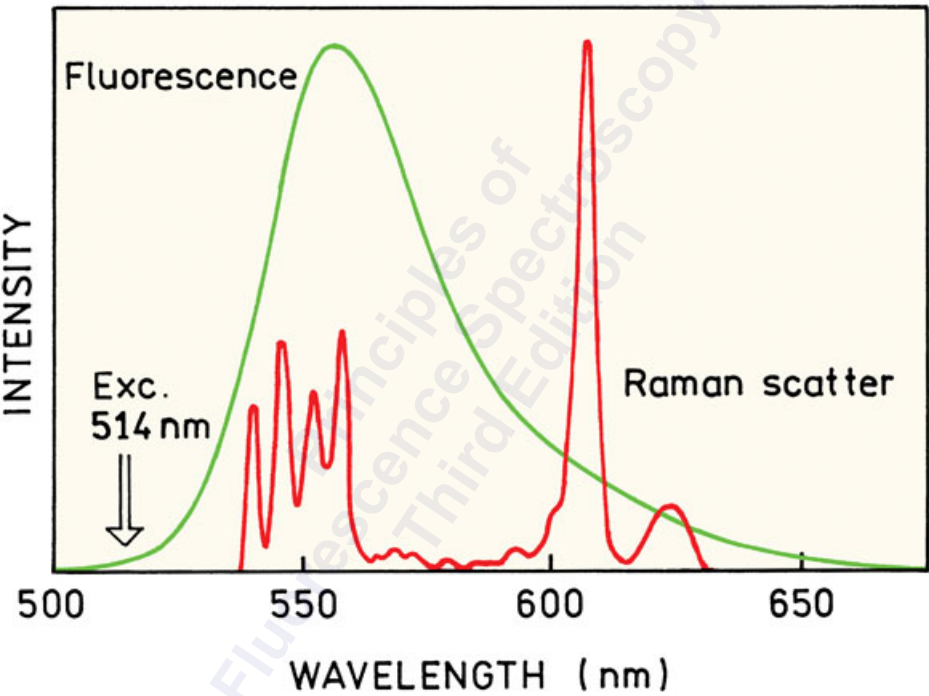
Add  $S-Q$

Ensemble Measurement

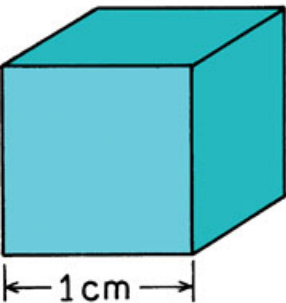


Single Molecule Measurement





One R6G molecule

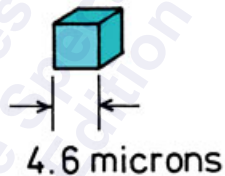


$$I_F \approx 1.0$$

$$I_{RS} \approx 10^{10}$$

$$V = 1 \text{ ml}$$

One R6G

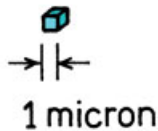


$$I_F \approx 1.0$$

$$I_{RS} \approx 1.0$$

$$V = 97 \text{ fl}$$

One R6G

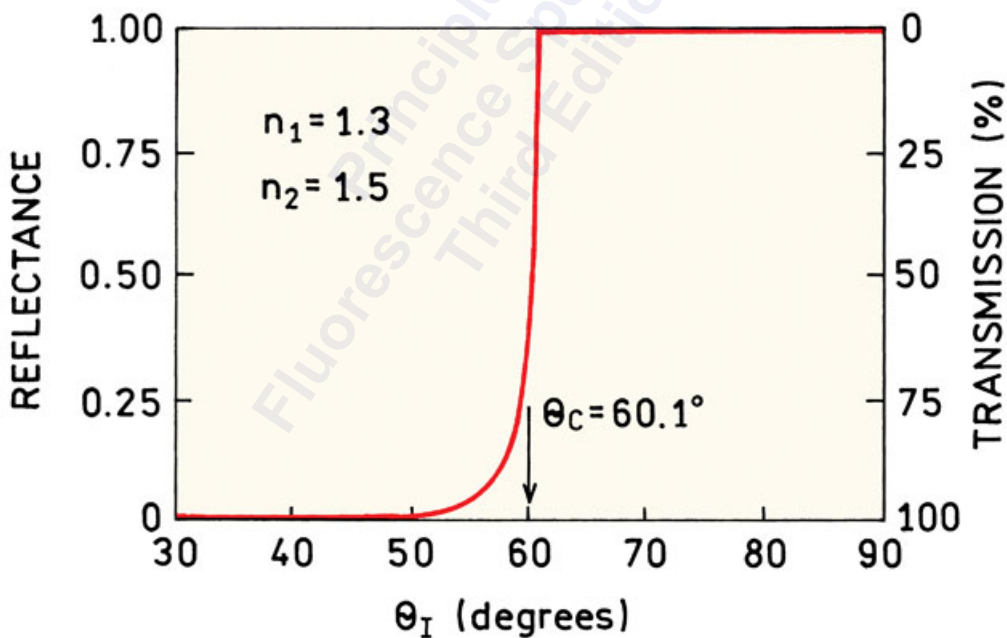
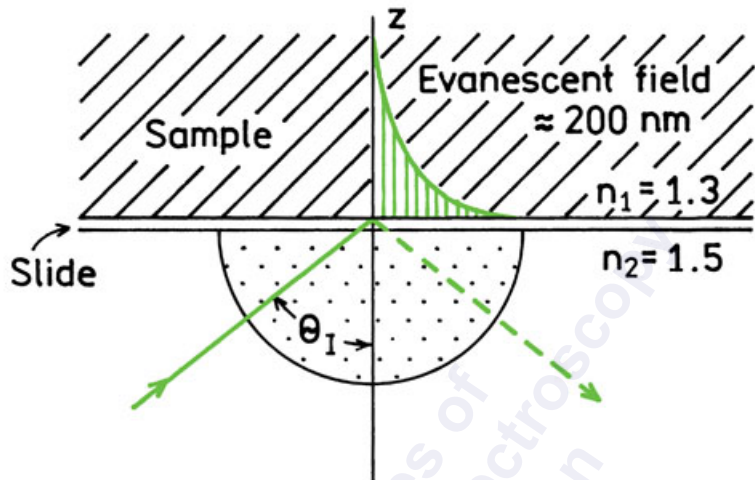


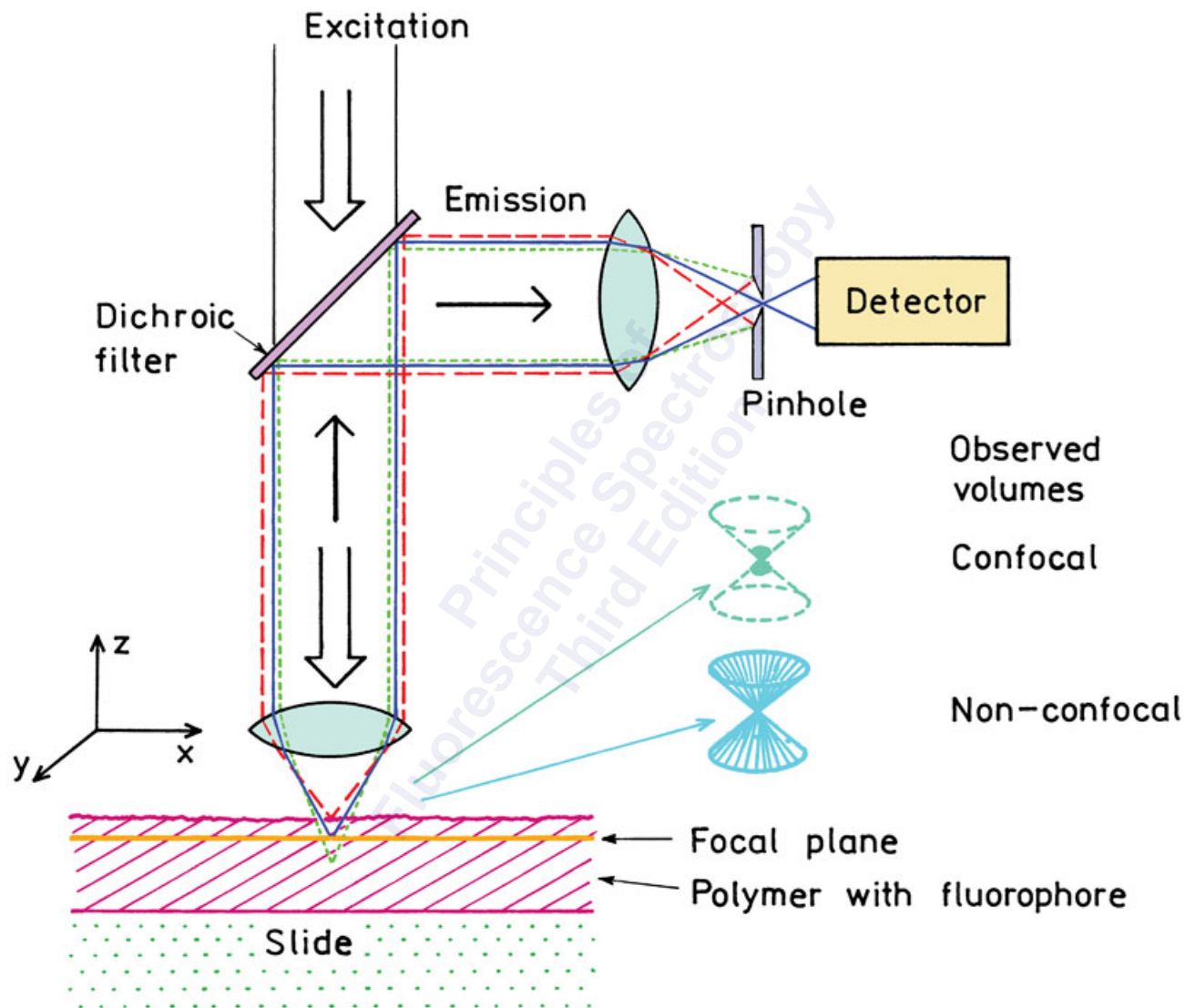
$$I_F \approx 1.0$$

$$I_{RS} \approx 10^{-2}$$

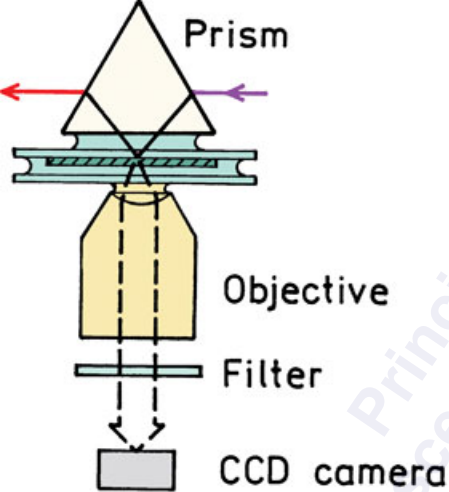
$$V = 1 \text{ fl}$$



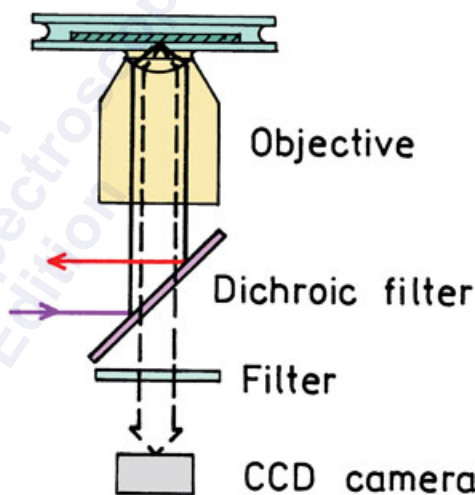




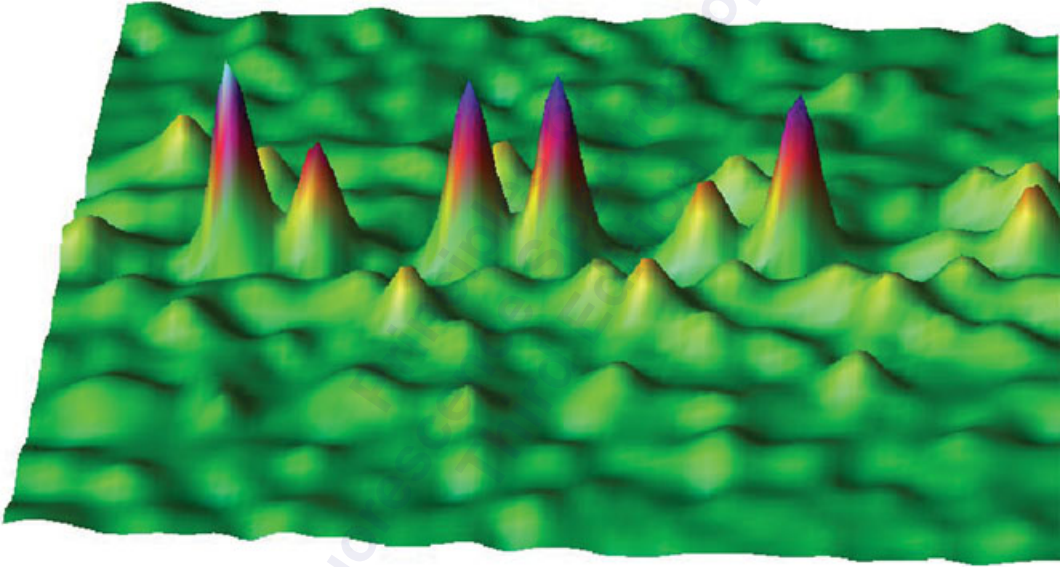


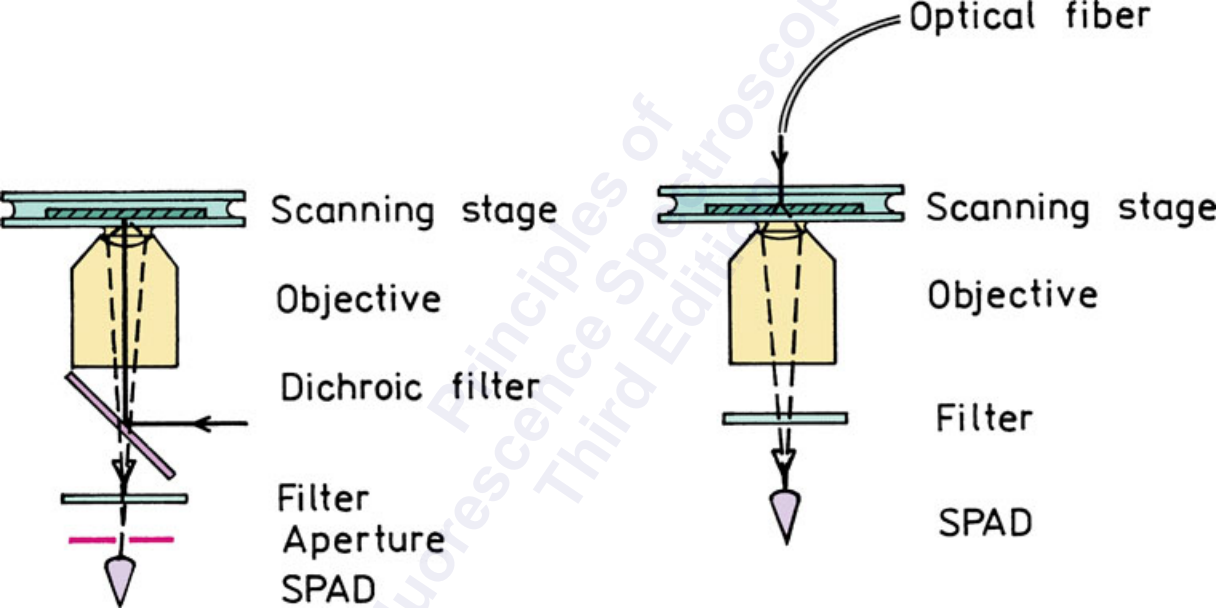


Prism - TIR

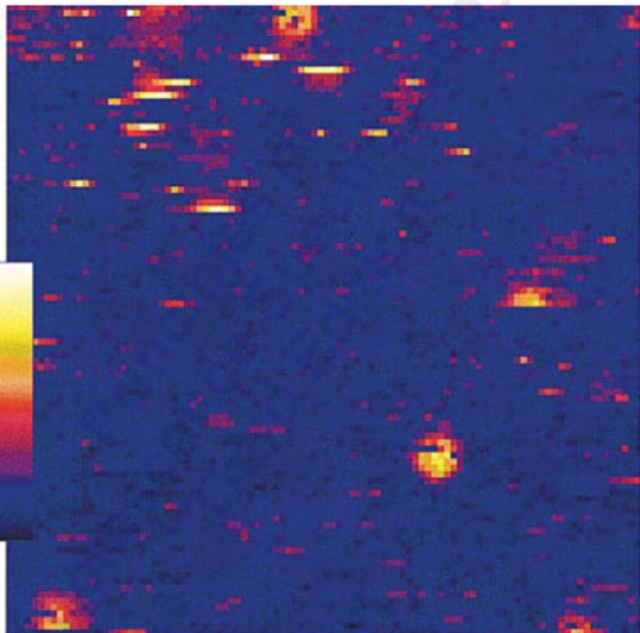


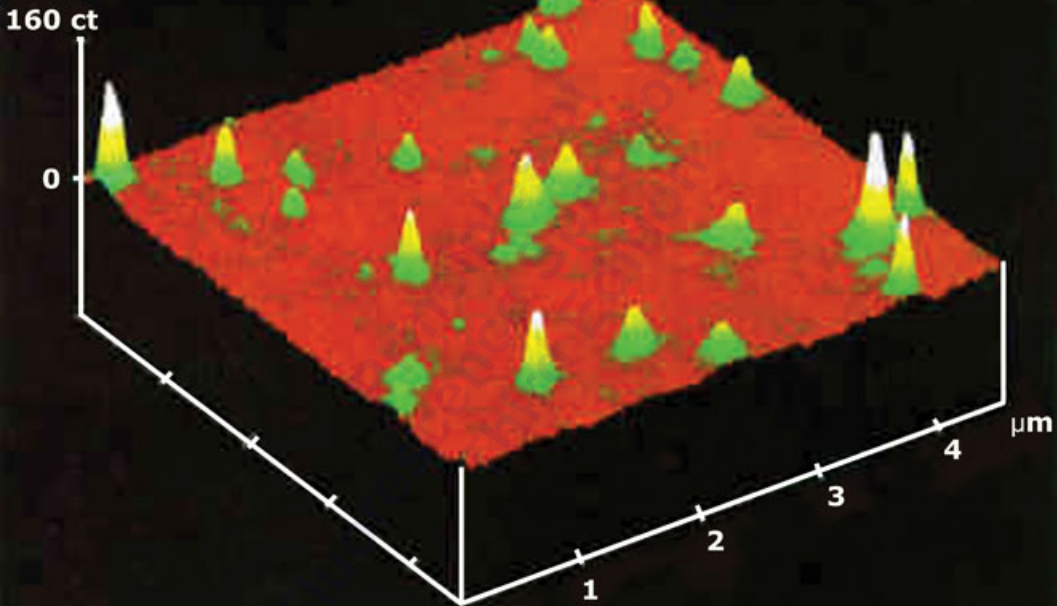
Objective - TIR

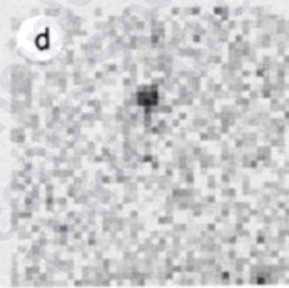
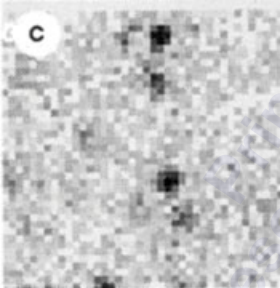
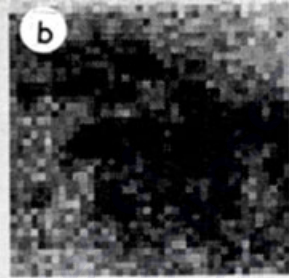
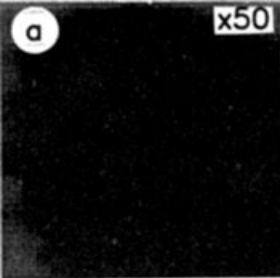




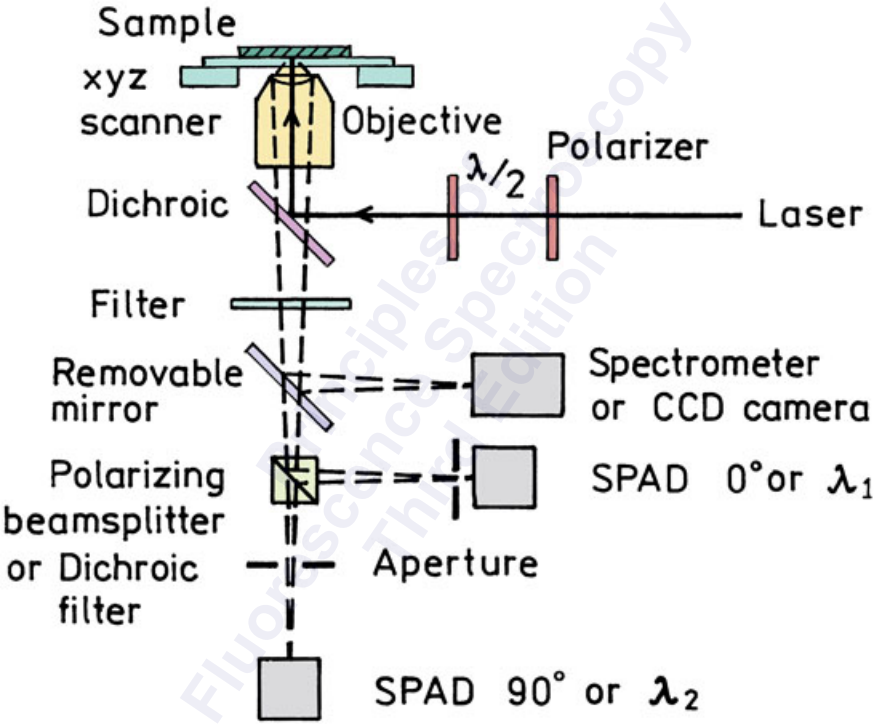
2.0262  
1.6382  
1.25021  
0.862208  
0.47421  
0.086213

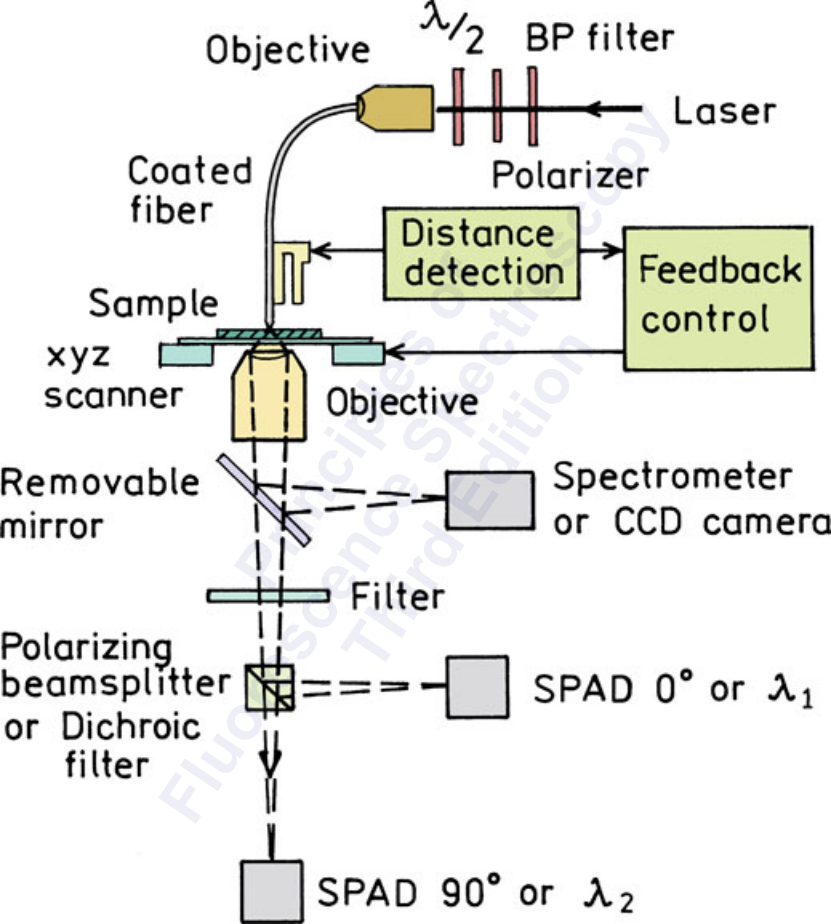




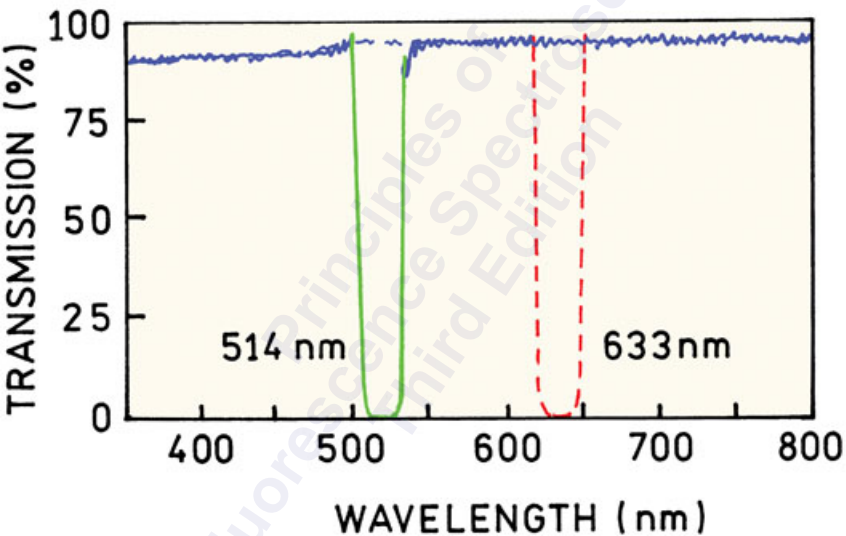


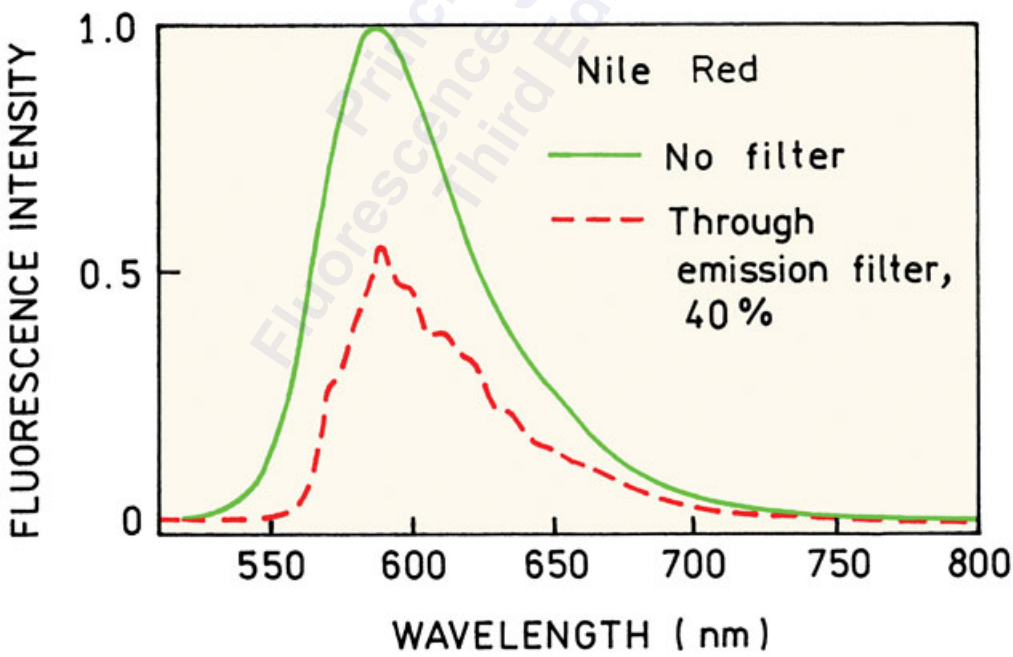
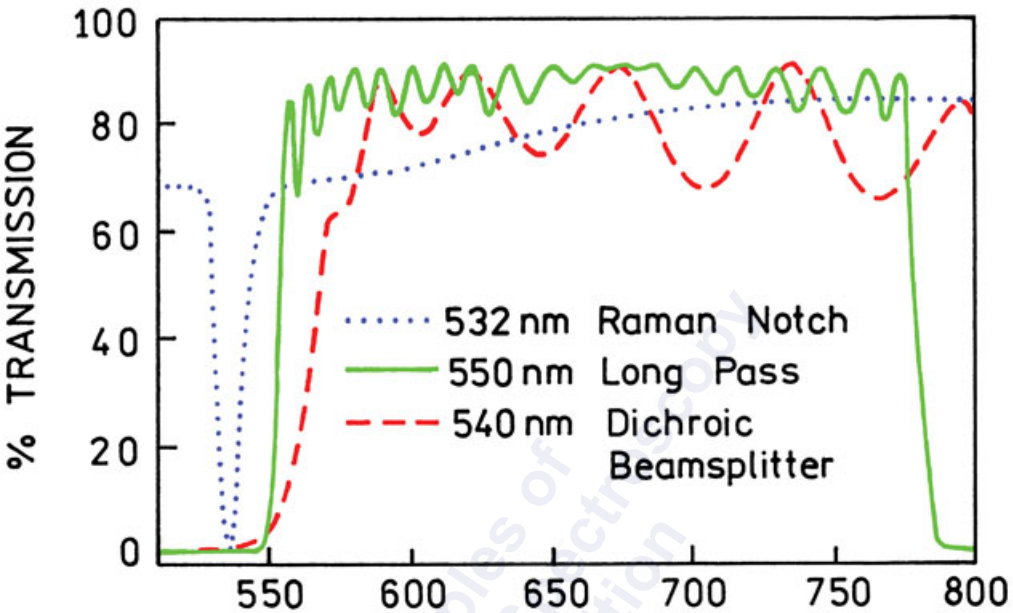
5  $\mu\text{m}$

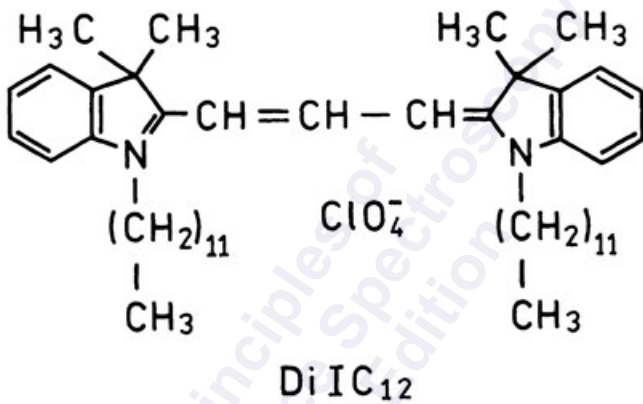


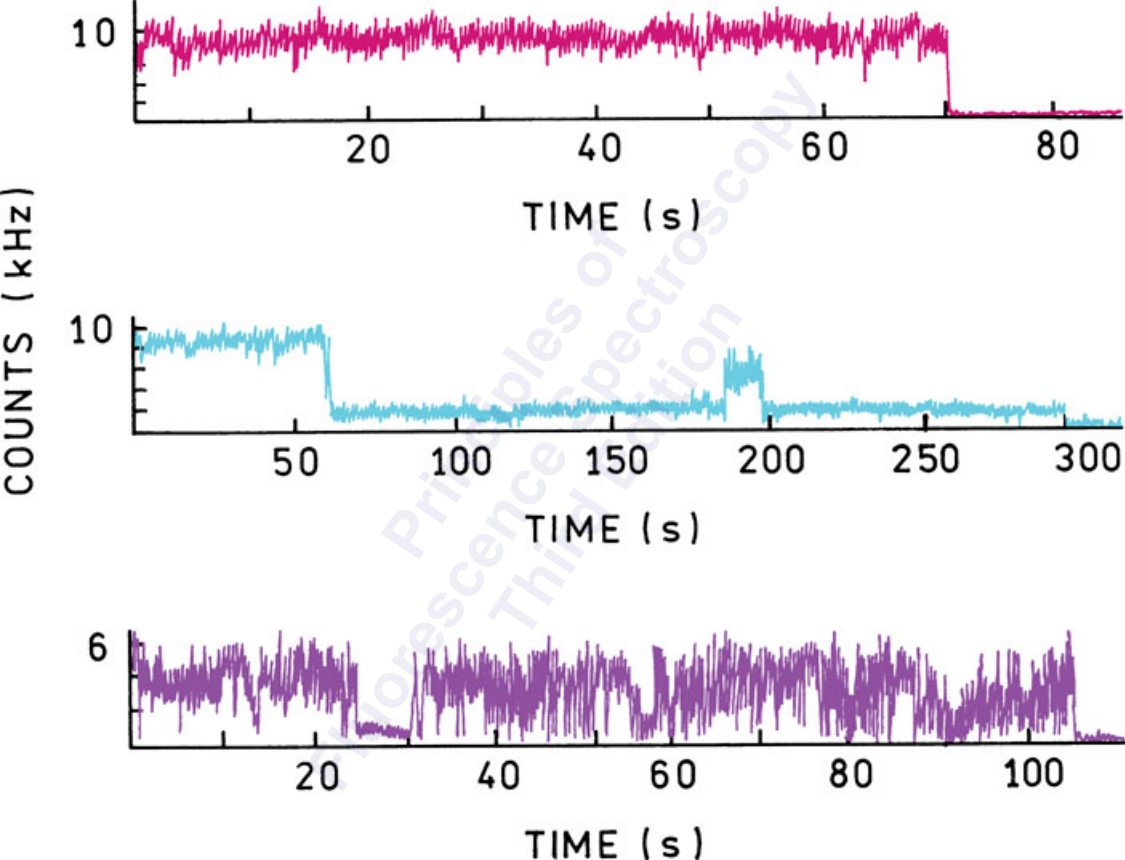


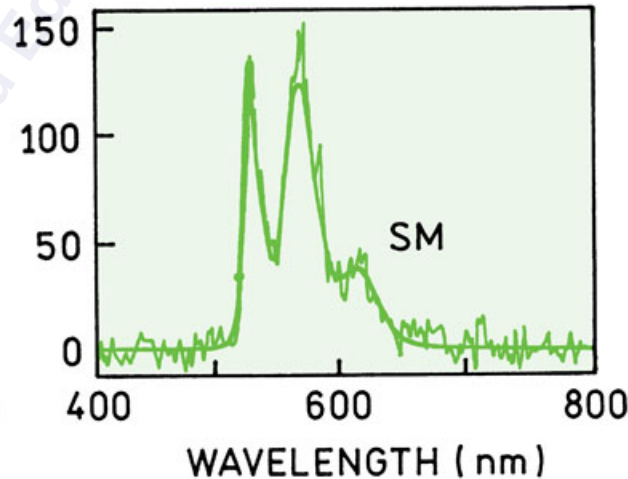
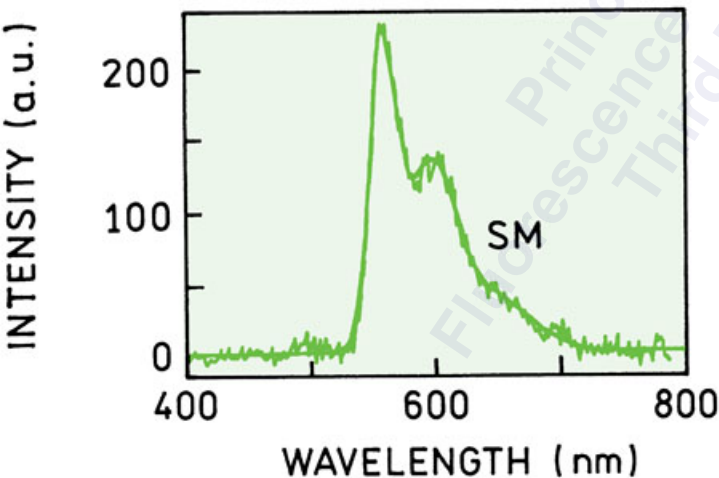
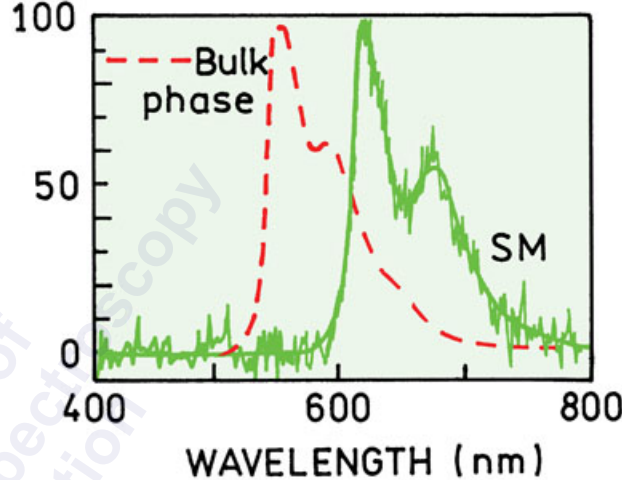
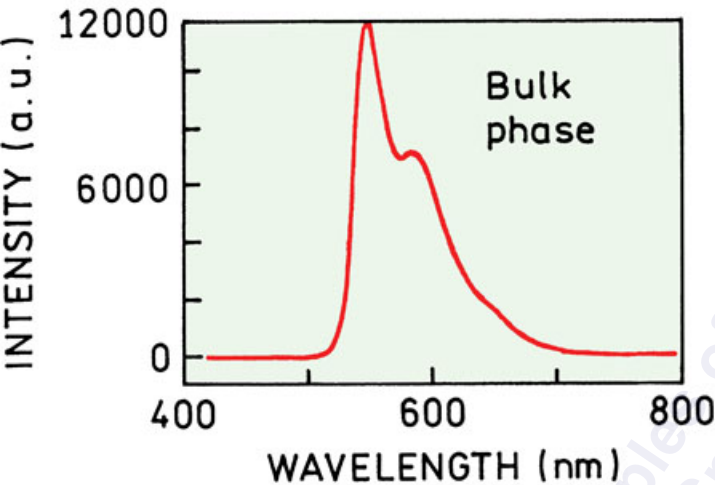


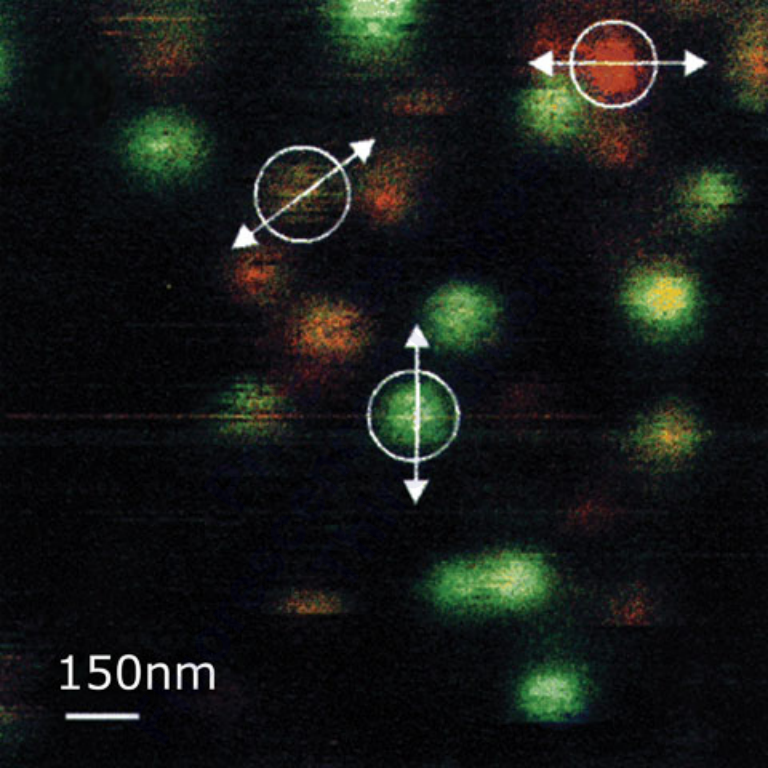




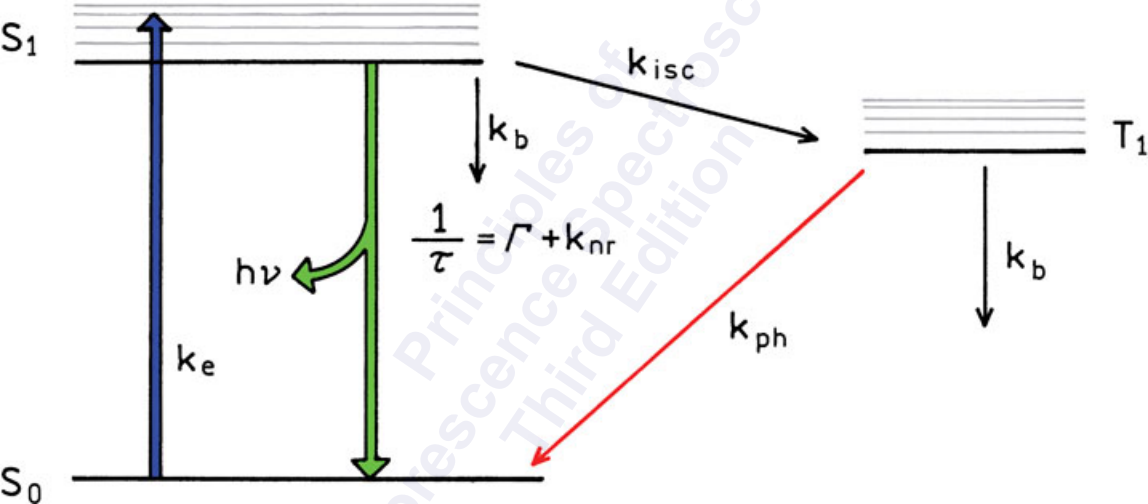








150nm



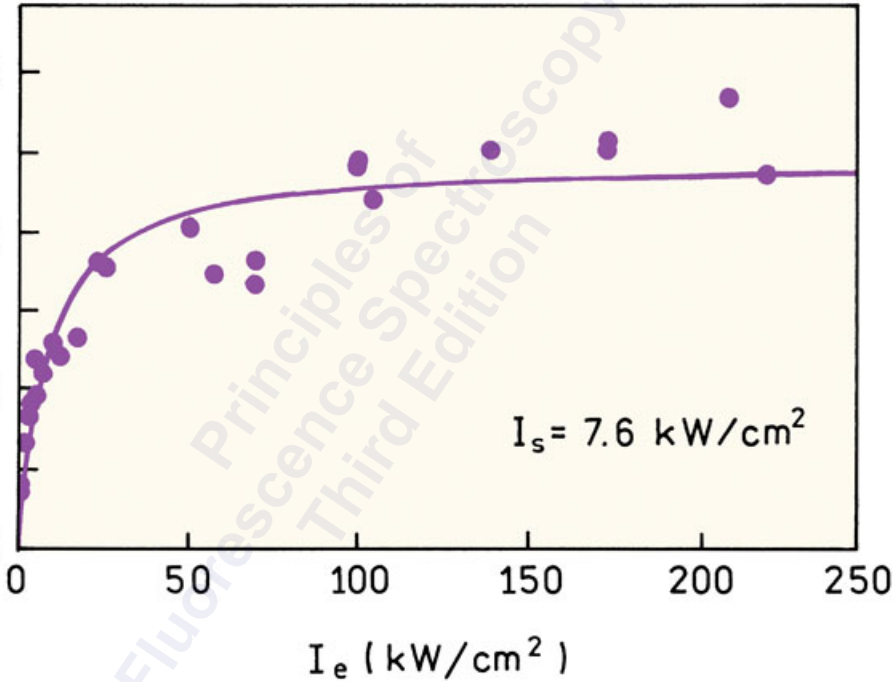
PHOTONS / RECORD

300

200

100

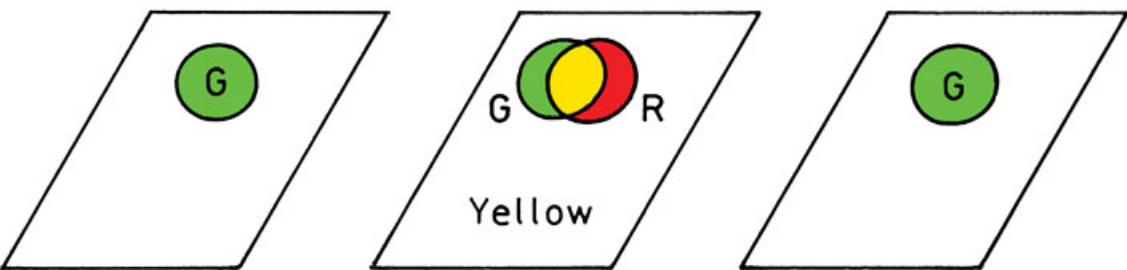
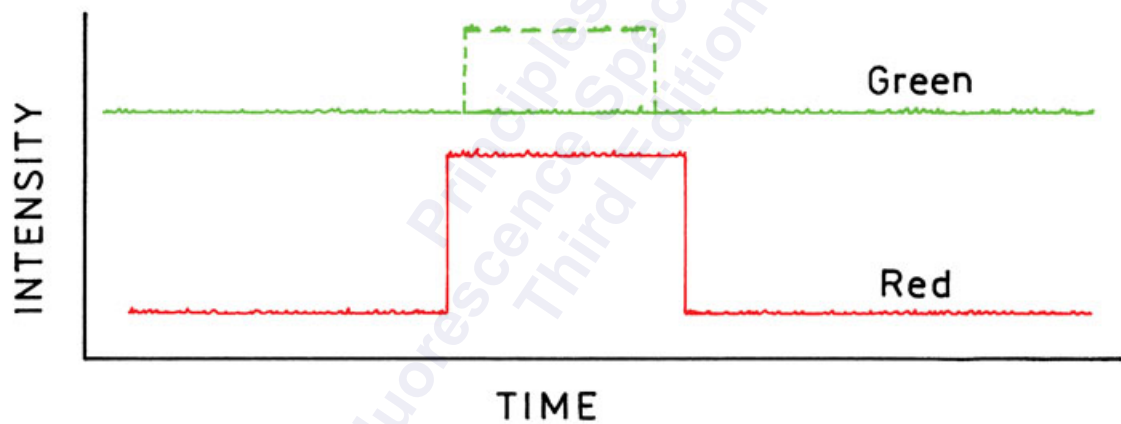
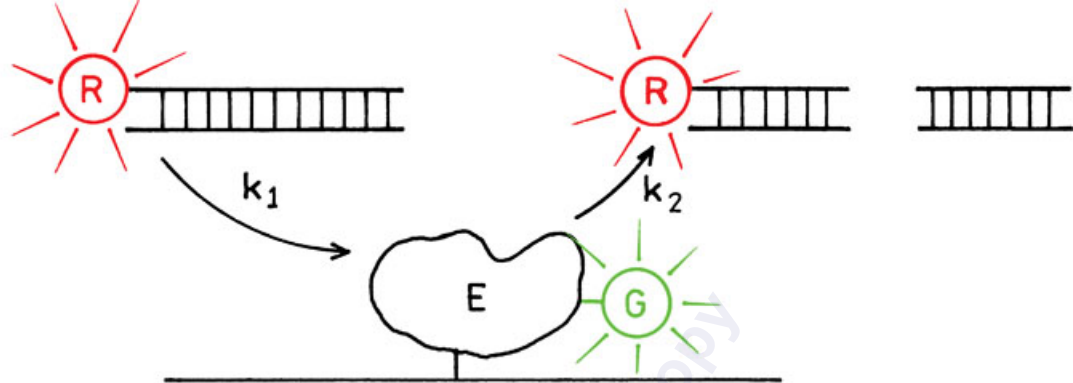
0



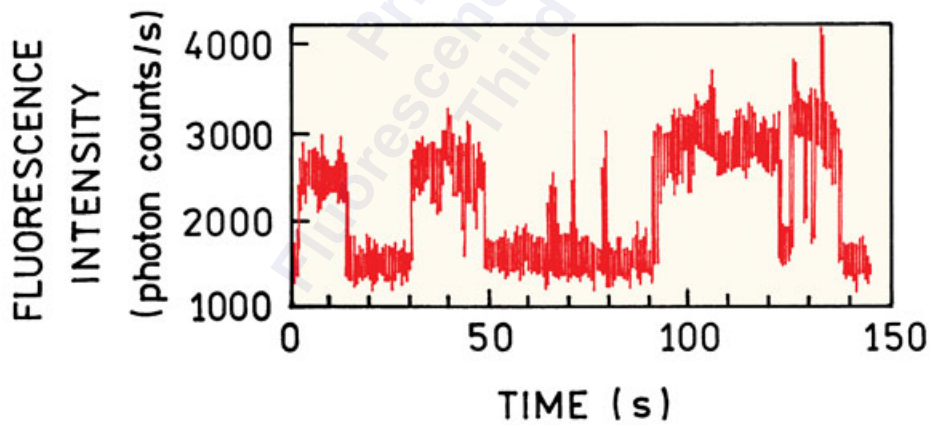
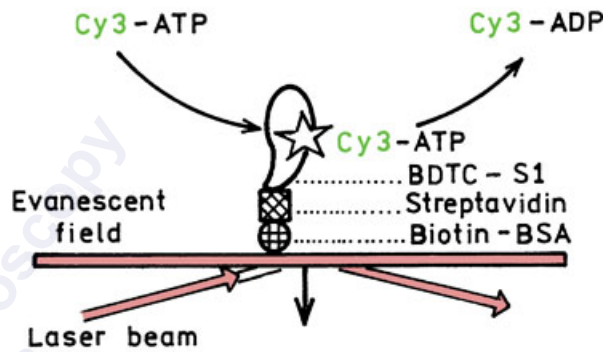
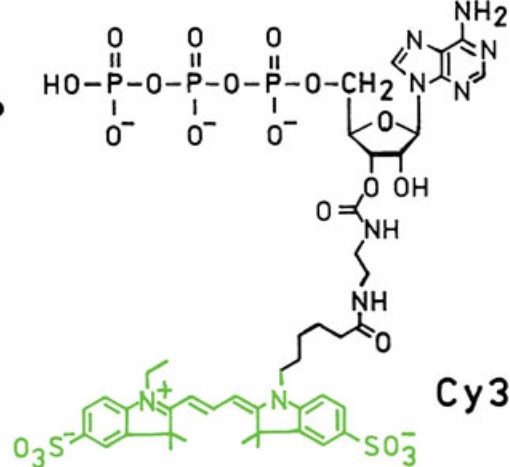
$I_e \text{ (kW/cm}^2\text{)}$

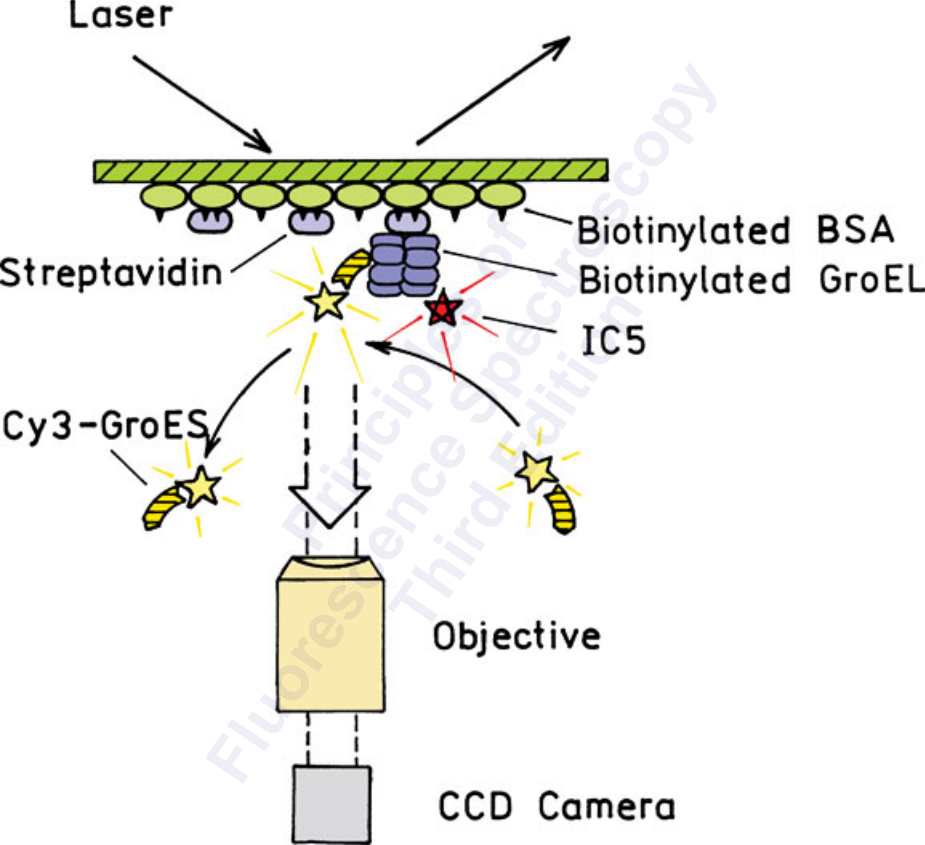
$I_s = 7.6 \text{ kW/cm}^2$

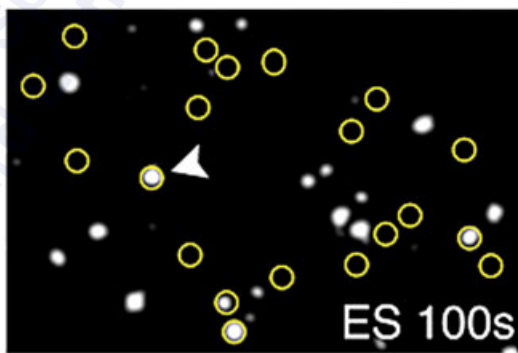
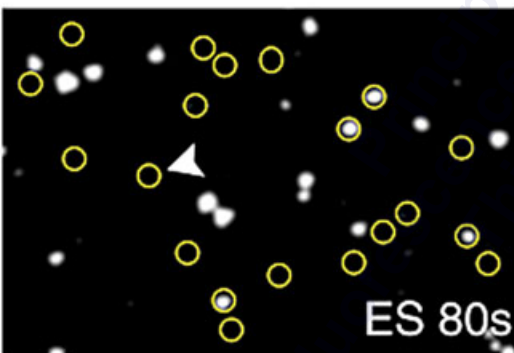
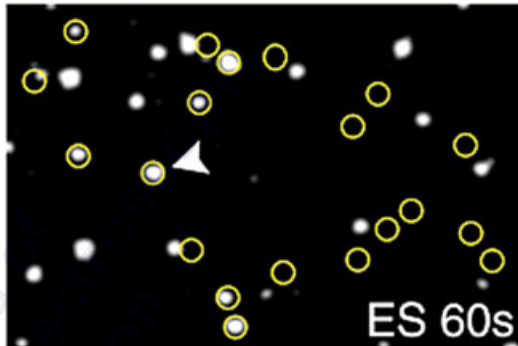




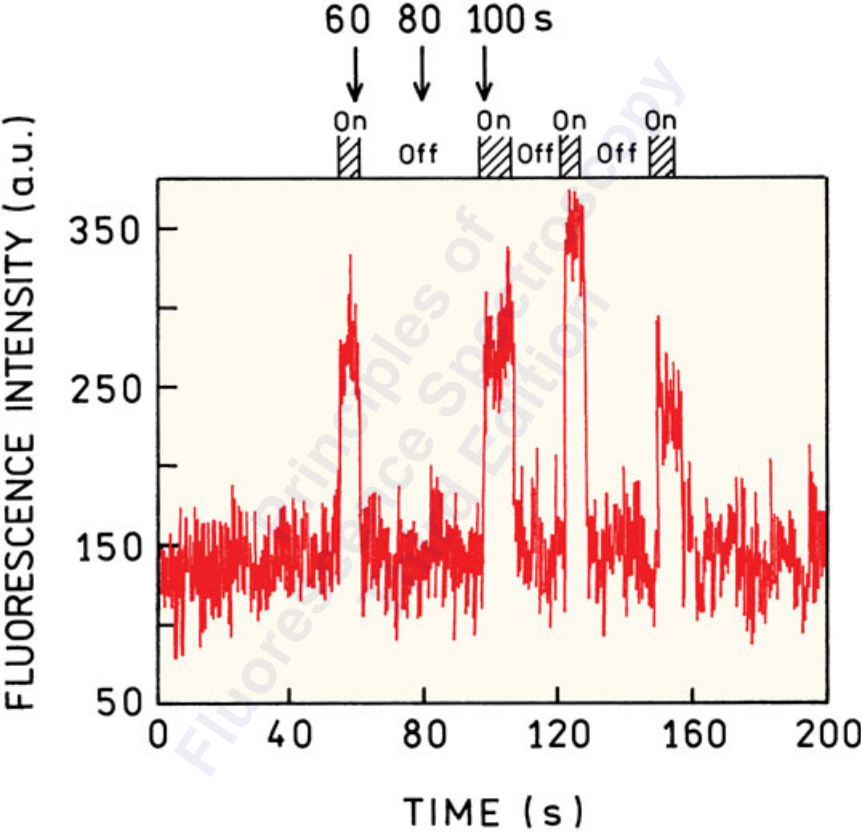
ATP

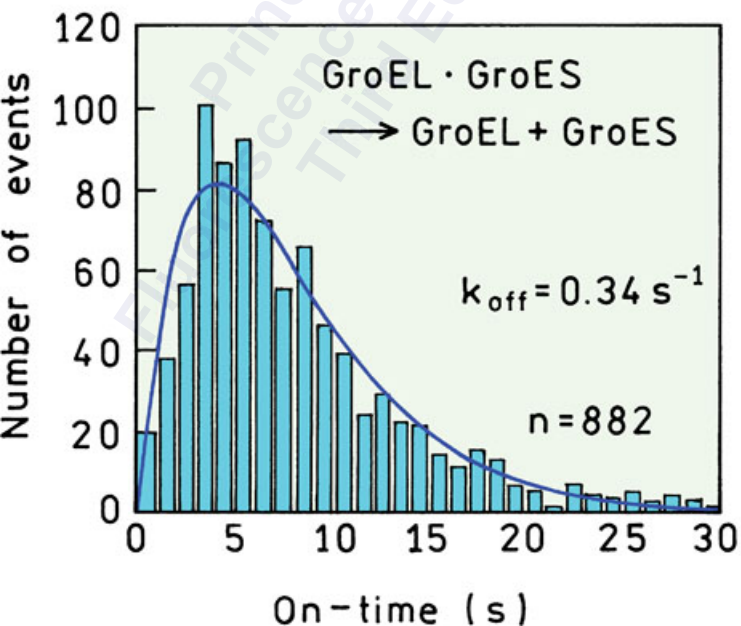
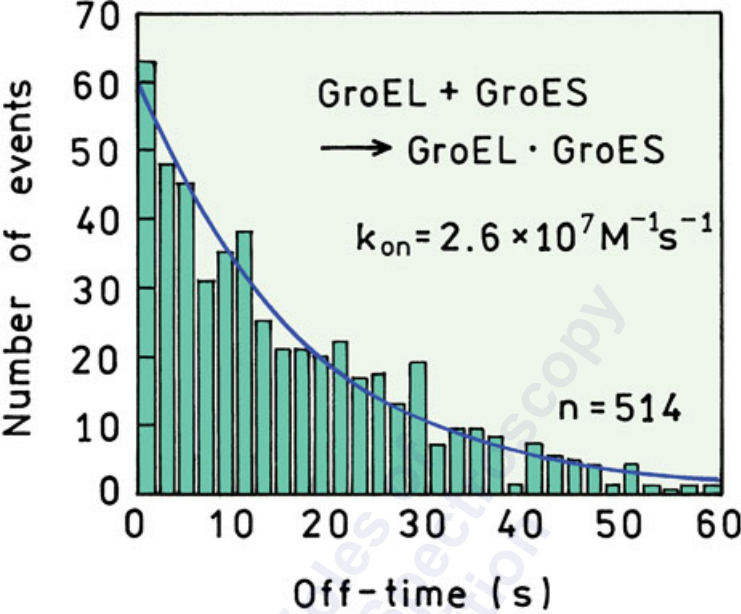


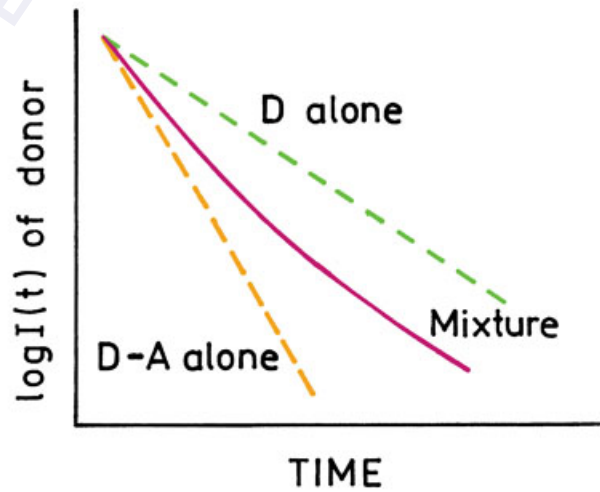
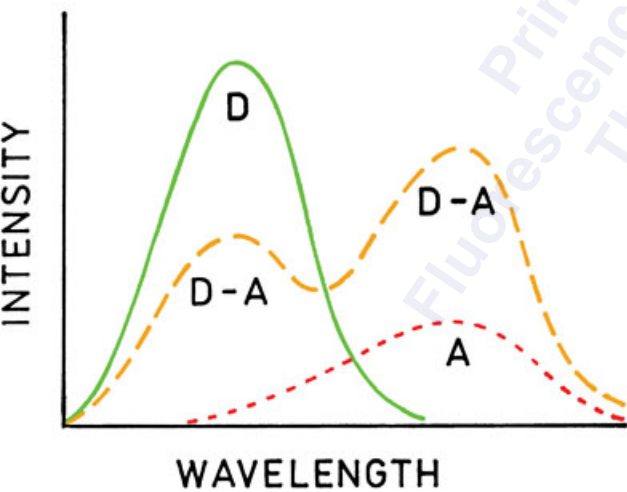
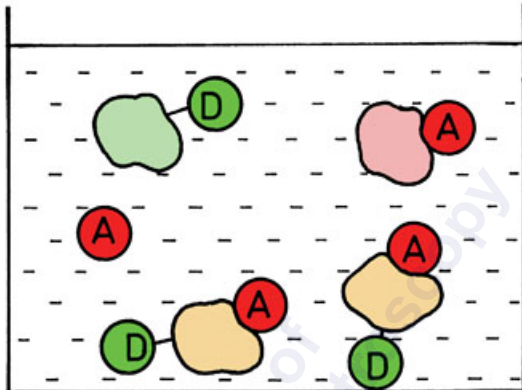


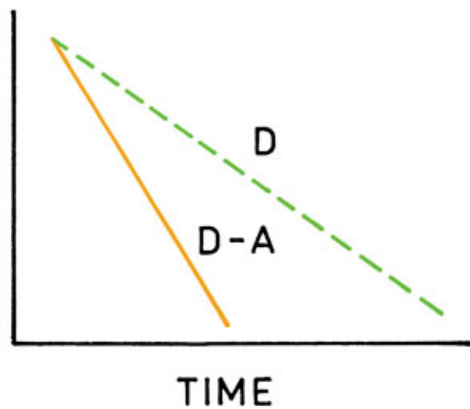
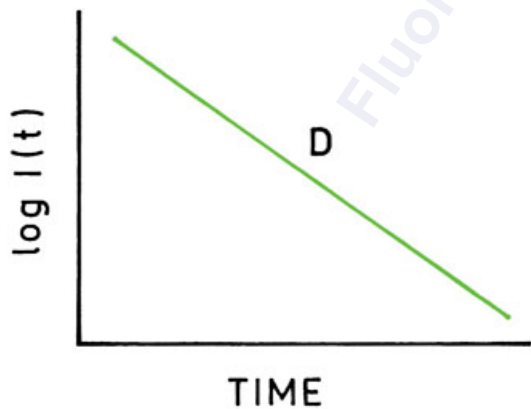
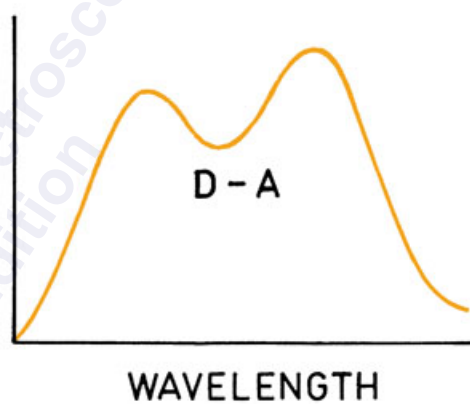
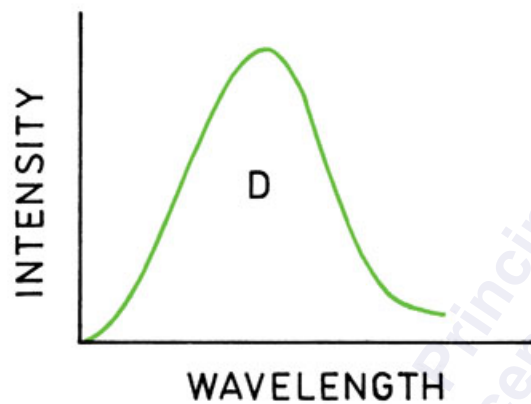
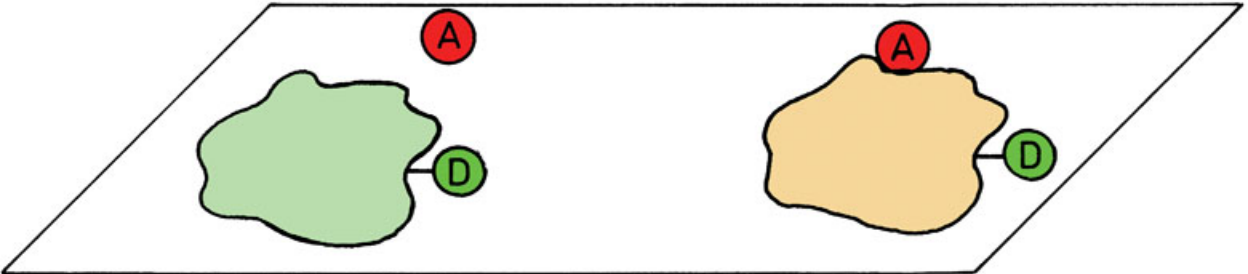


10  $\mu\text{m}$

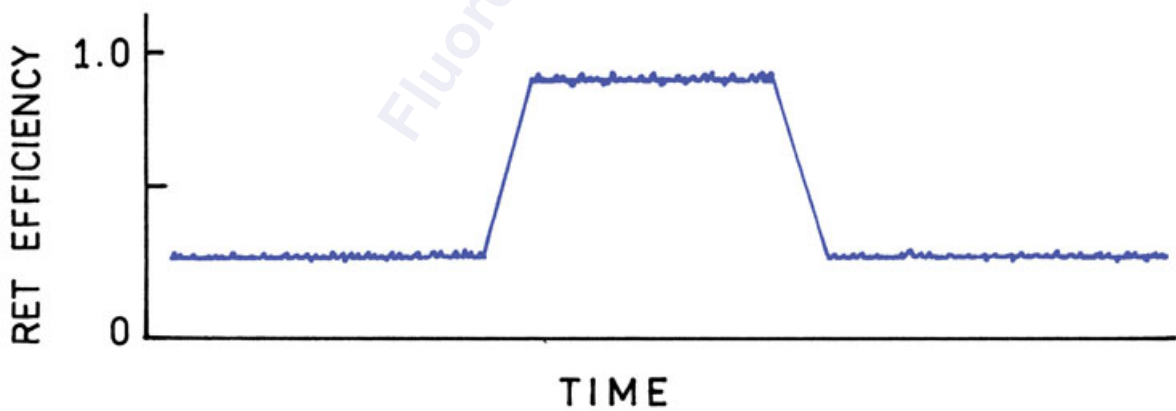
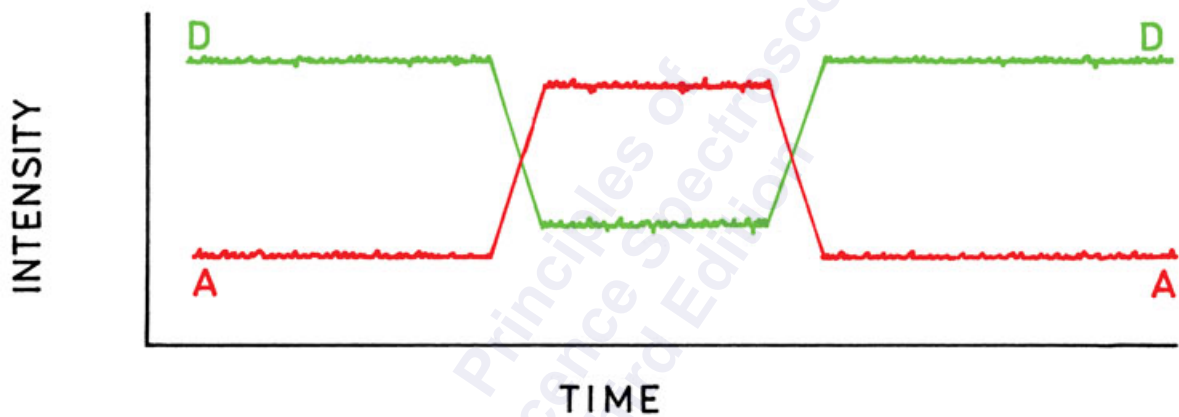
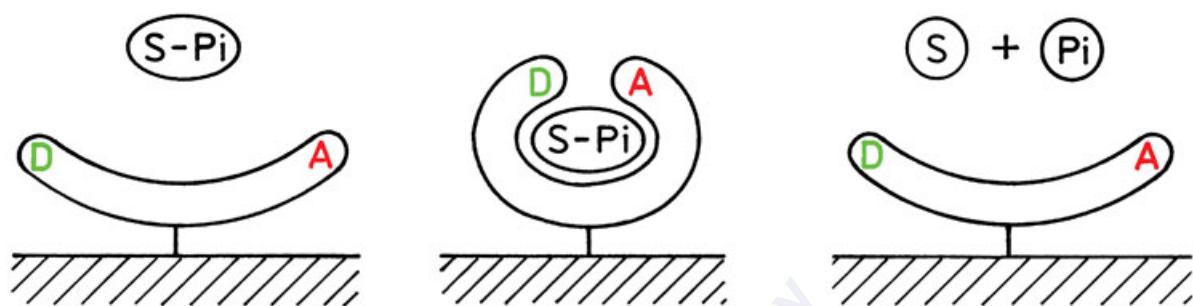






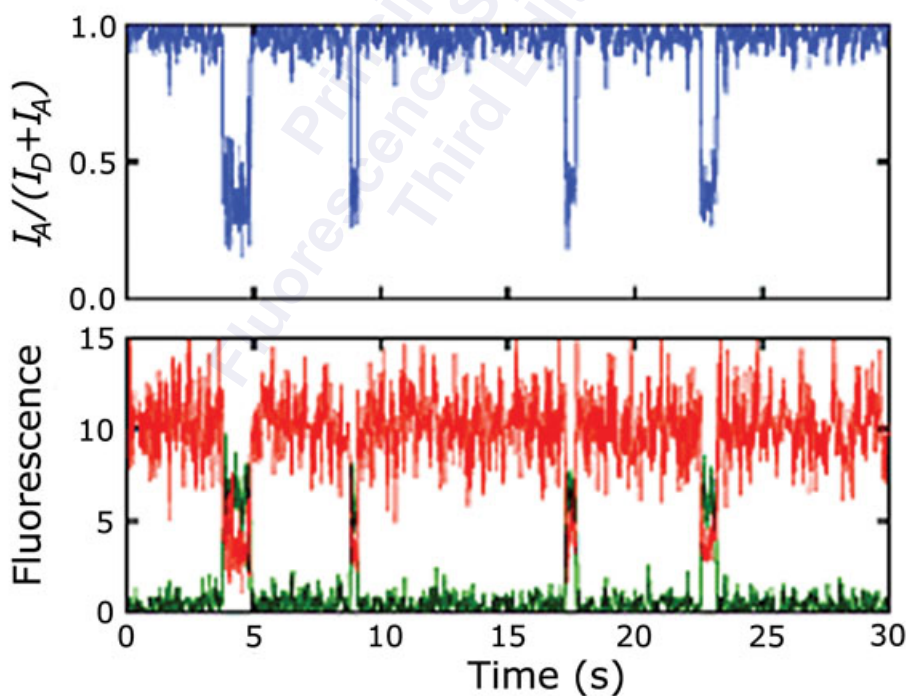
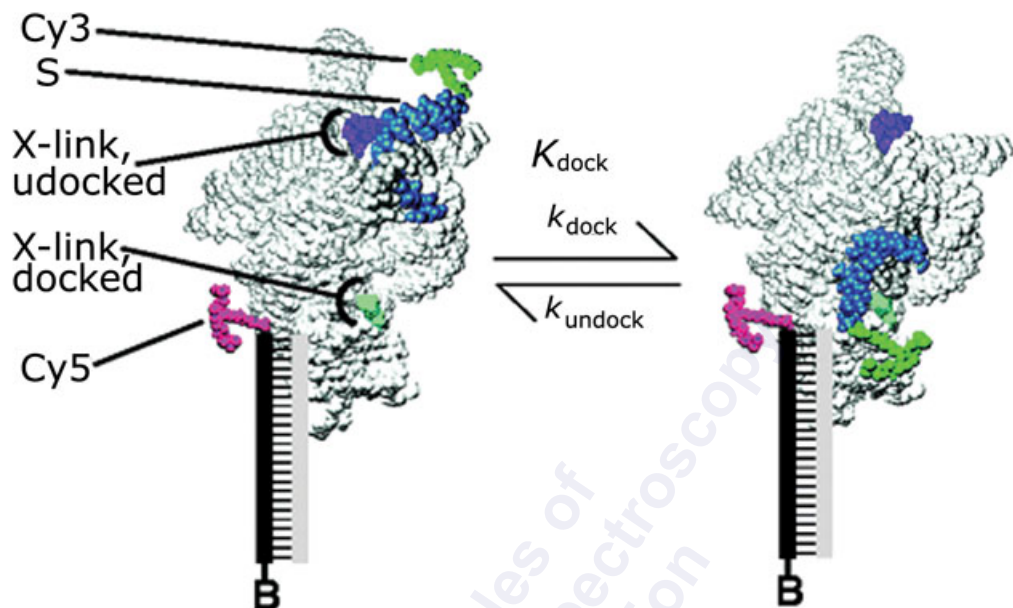


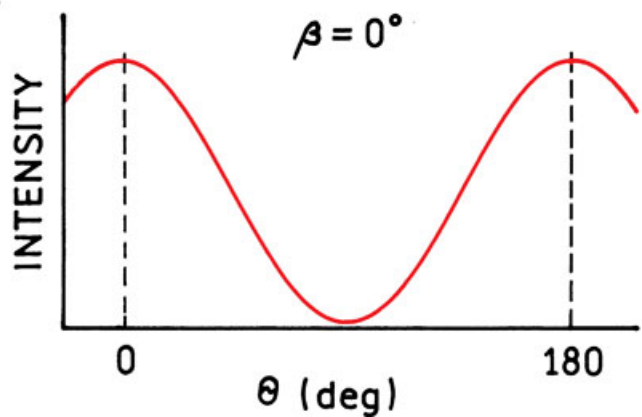
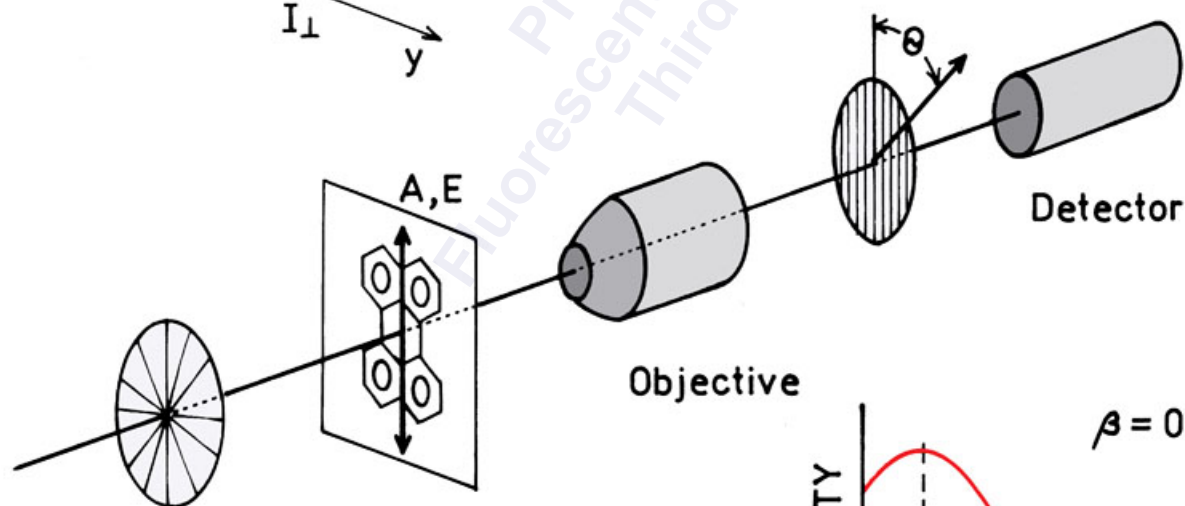
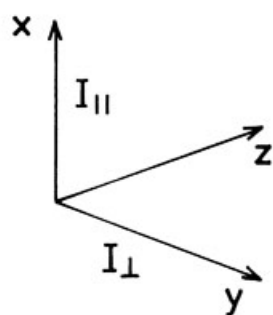
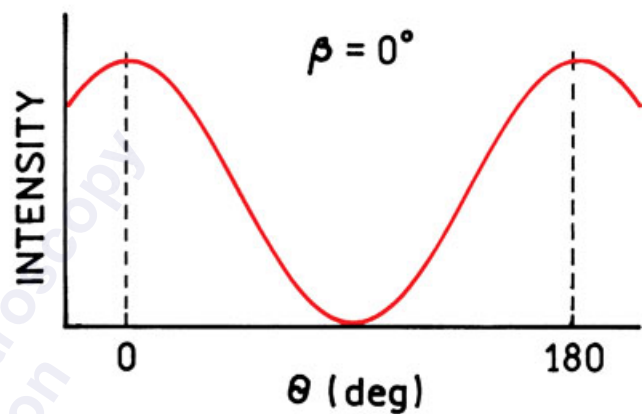
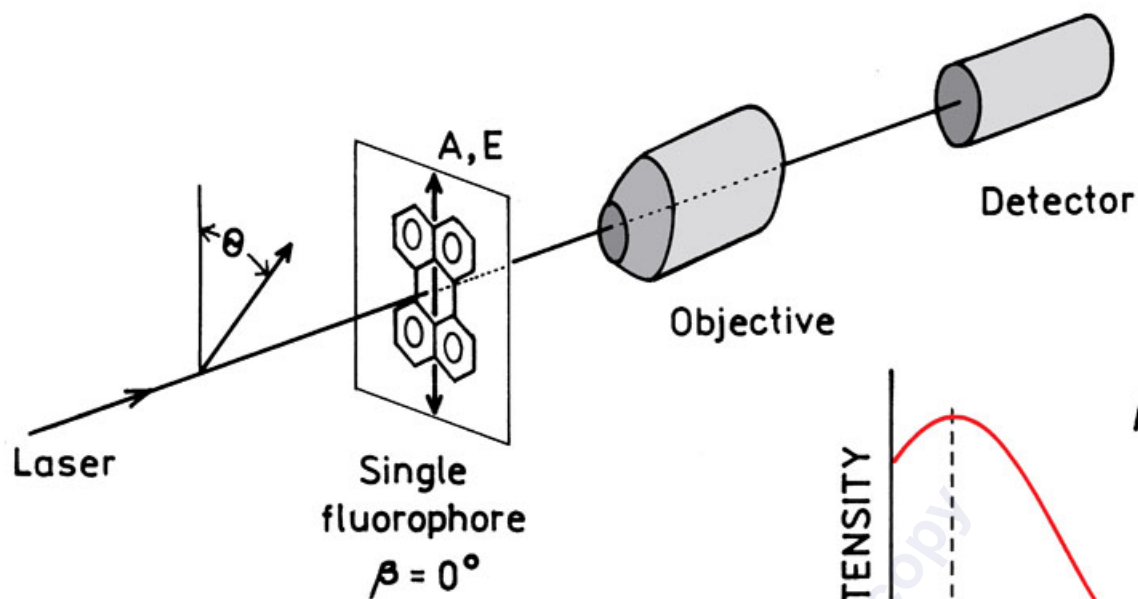


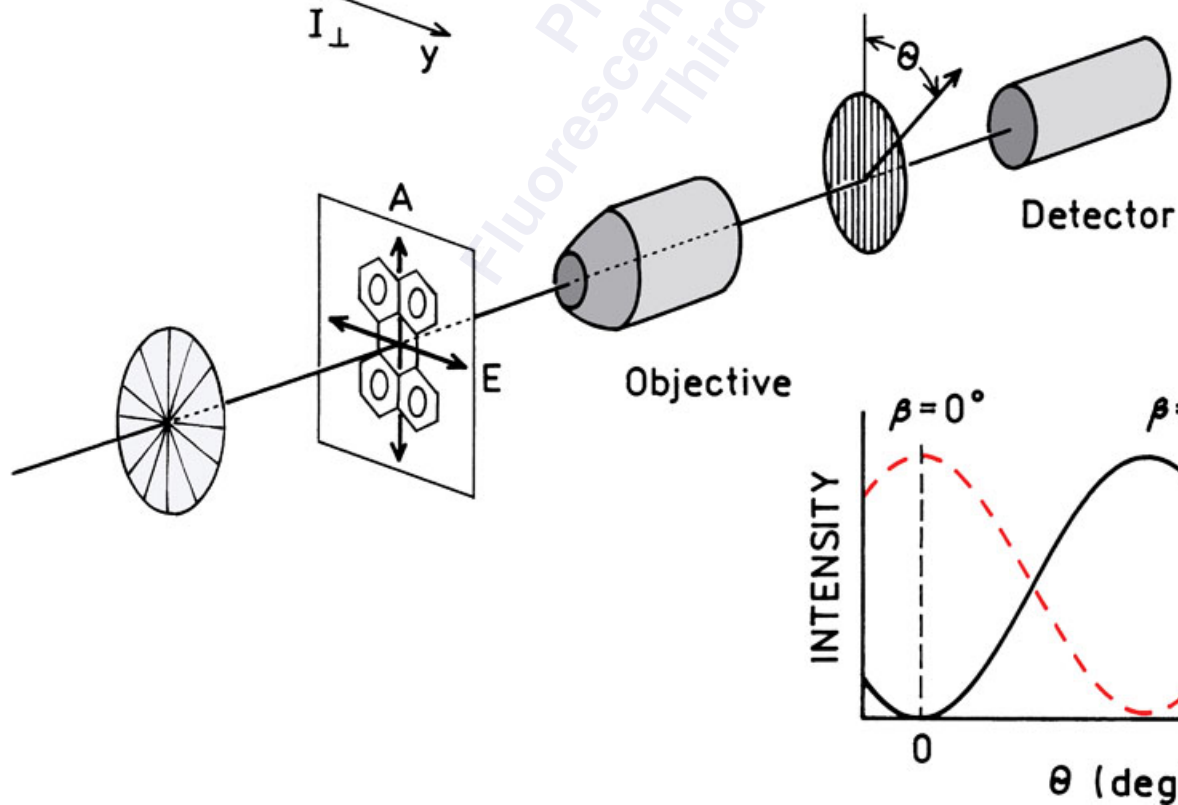
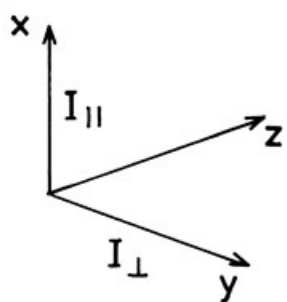
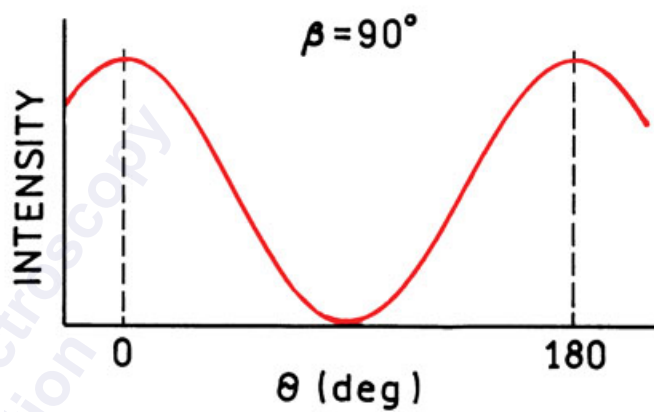
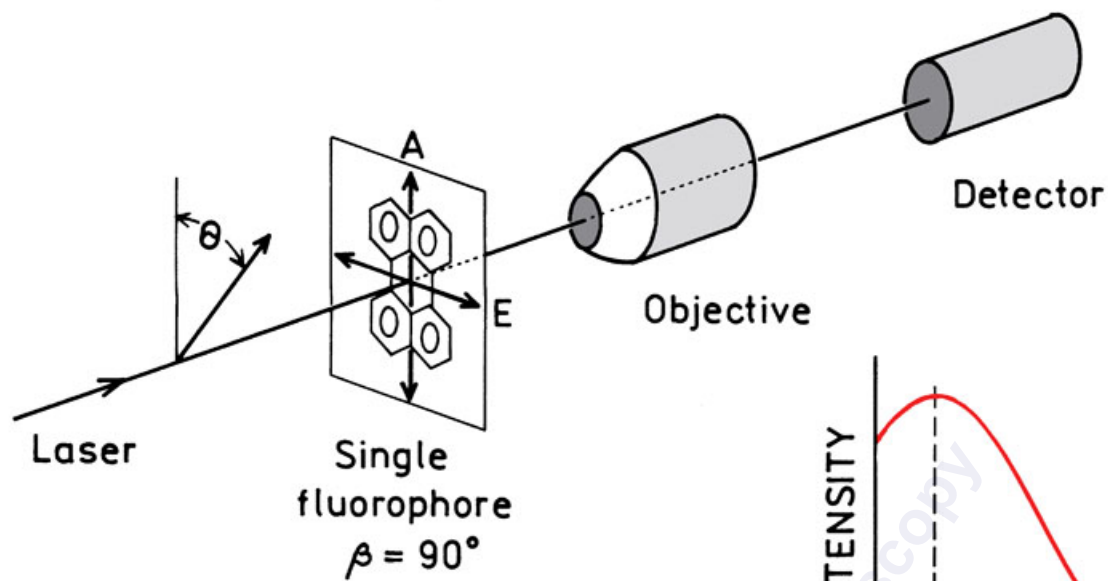


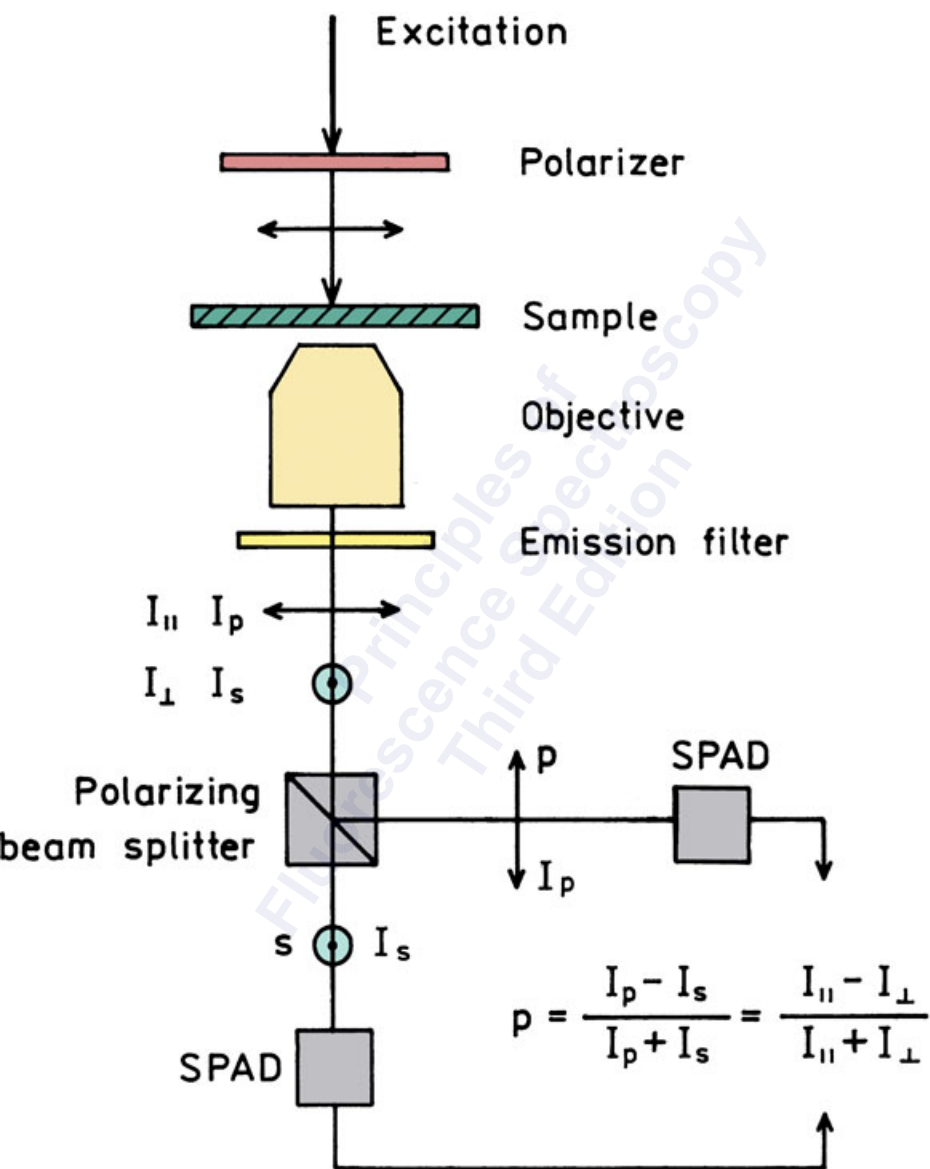
## P1 Undocked

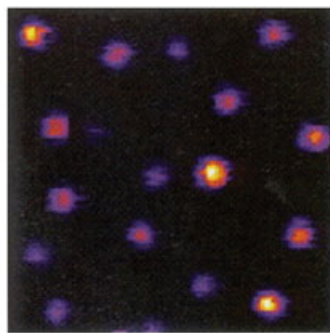
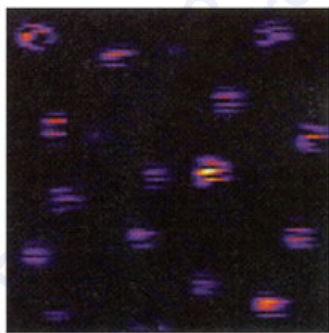
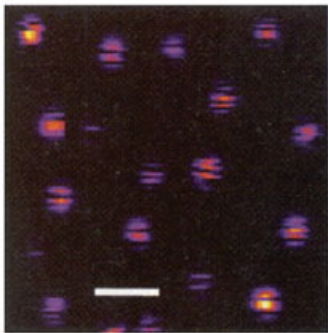
## P1 Docked

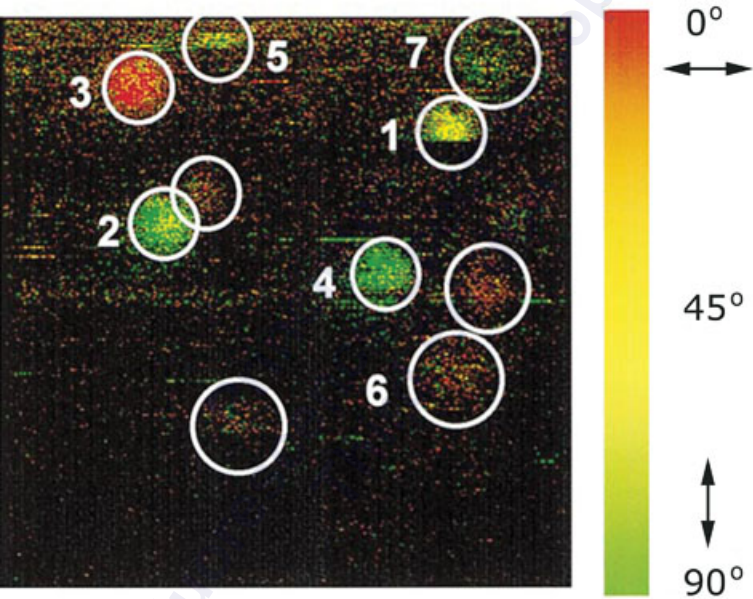




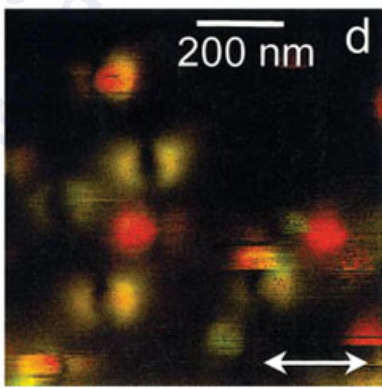
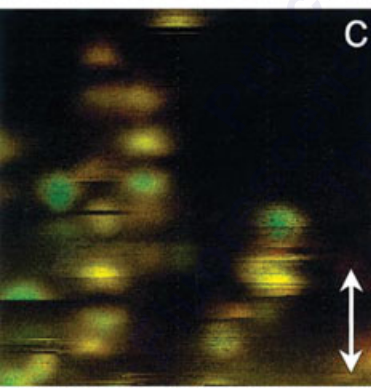
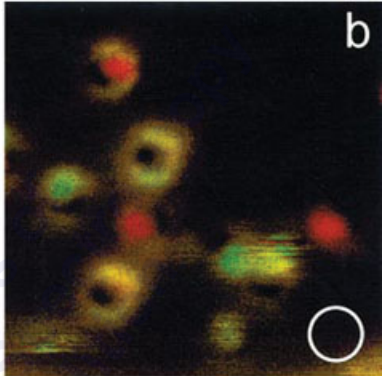
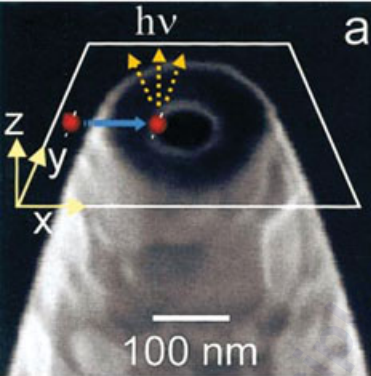




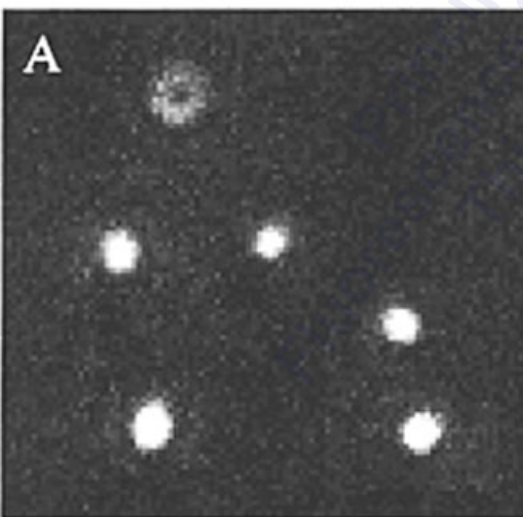
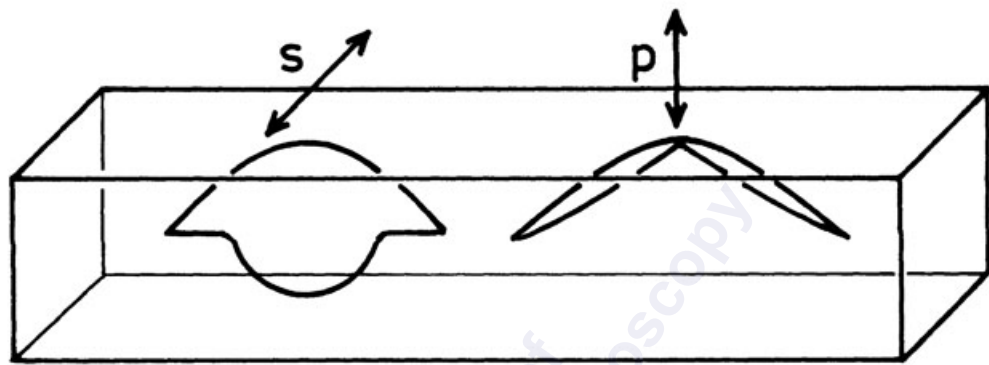




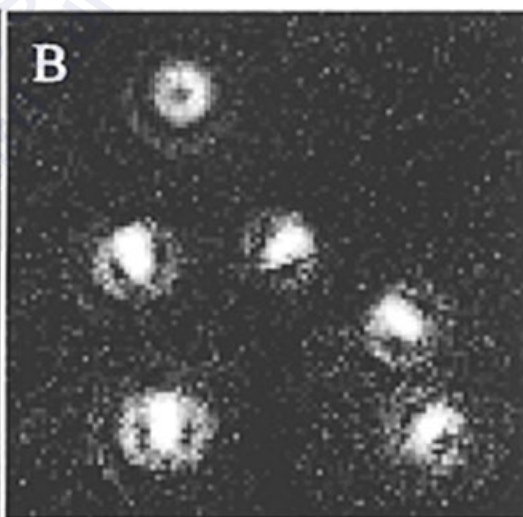








In-focus

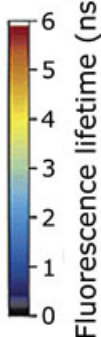
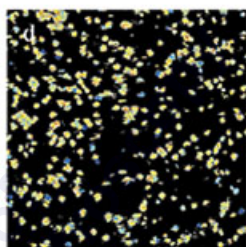
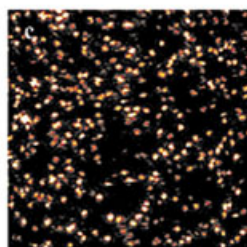
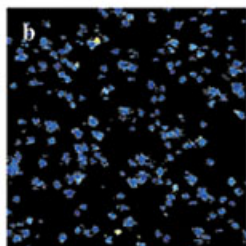
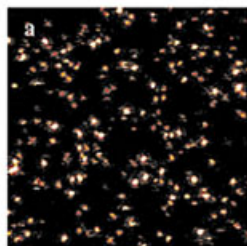
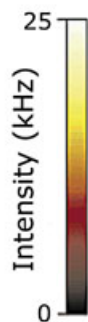


Defocused

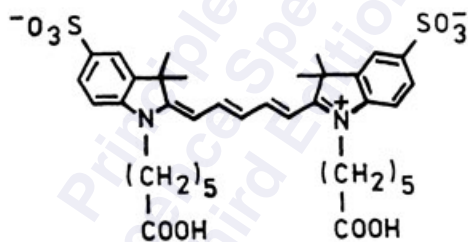
Intensity

Lifetimes

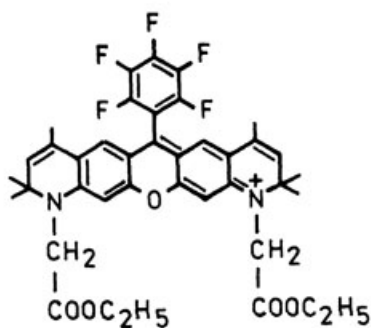
Cy5



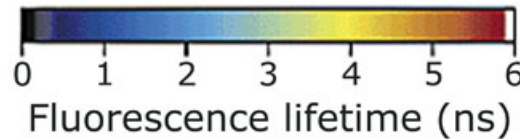
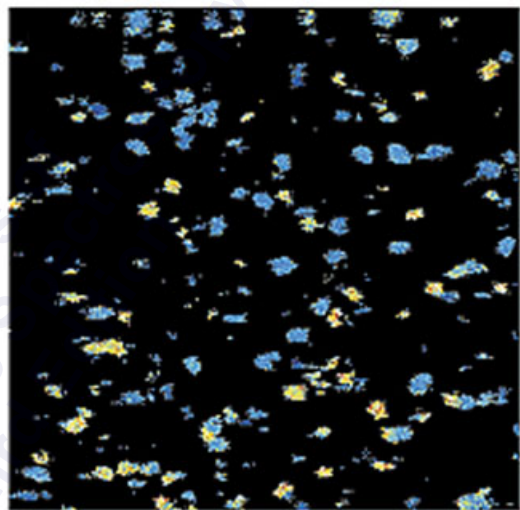
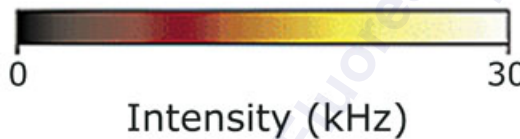
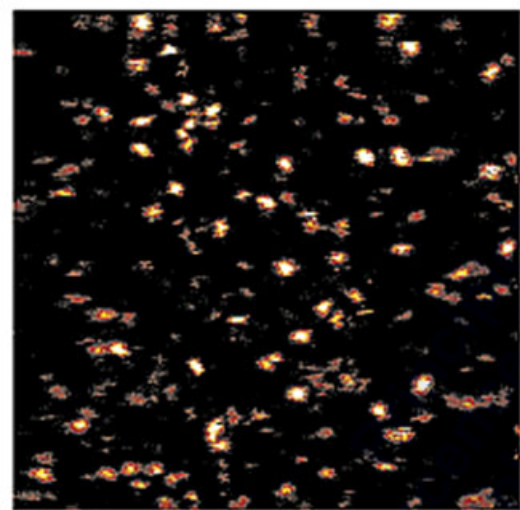
JF9

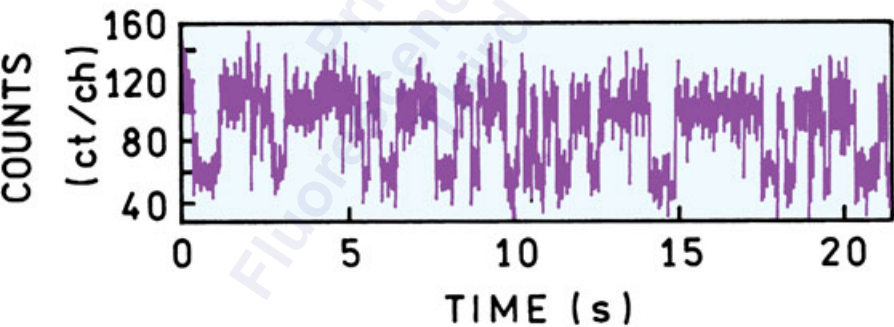
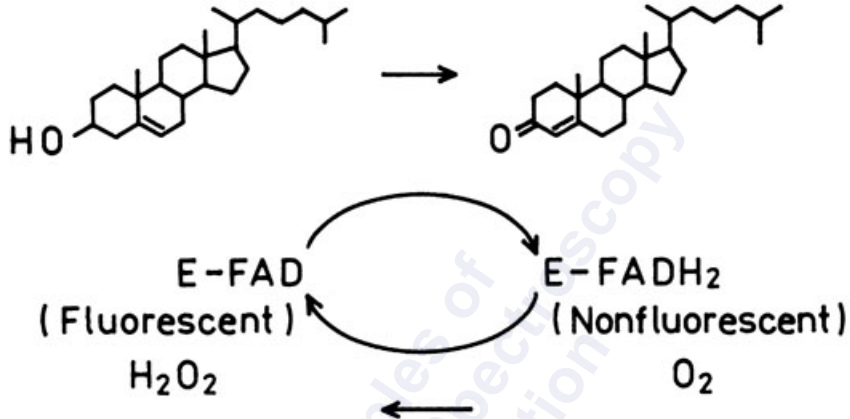


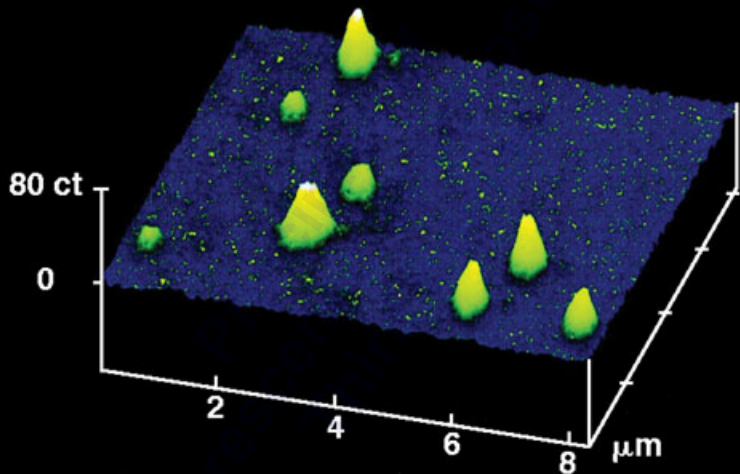
Cy 5

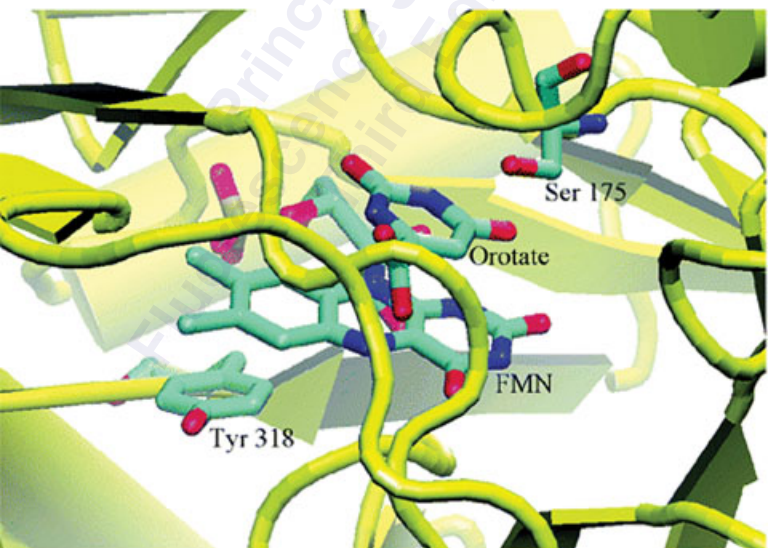
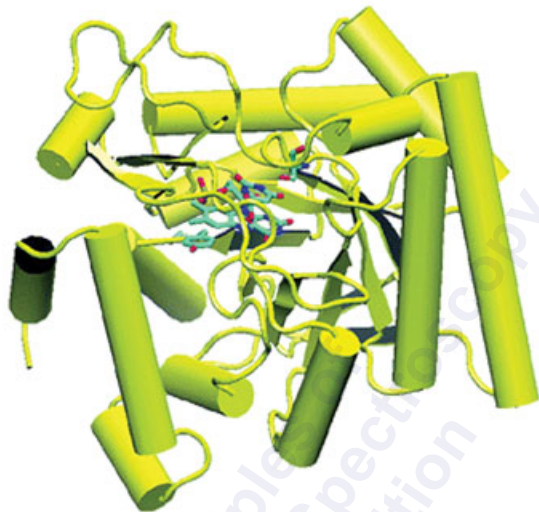


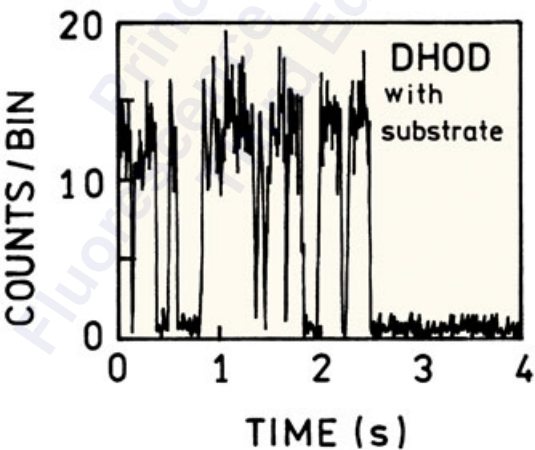
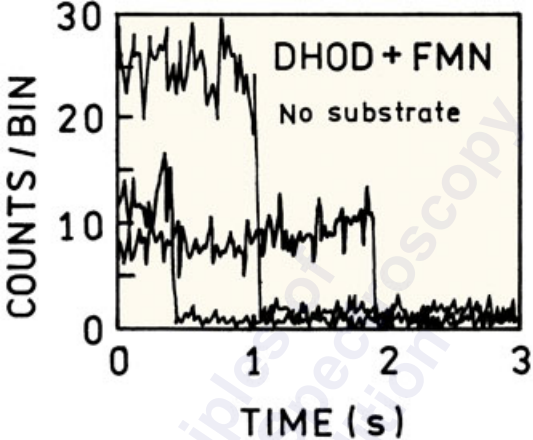
J F 9









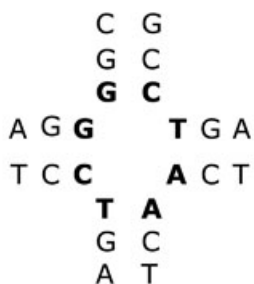




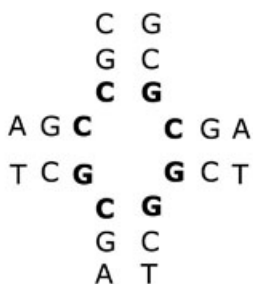




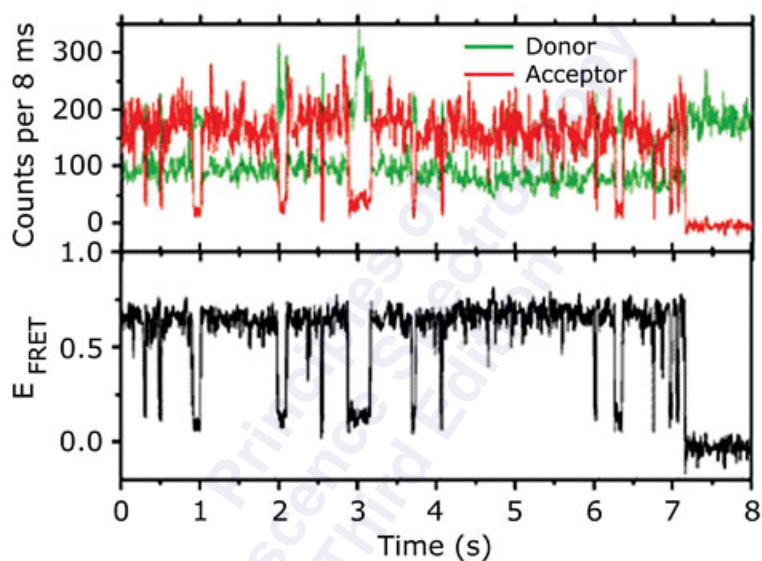
Junction 3



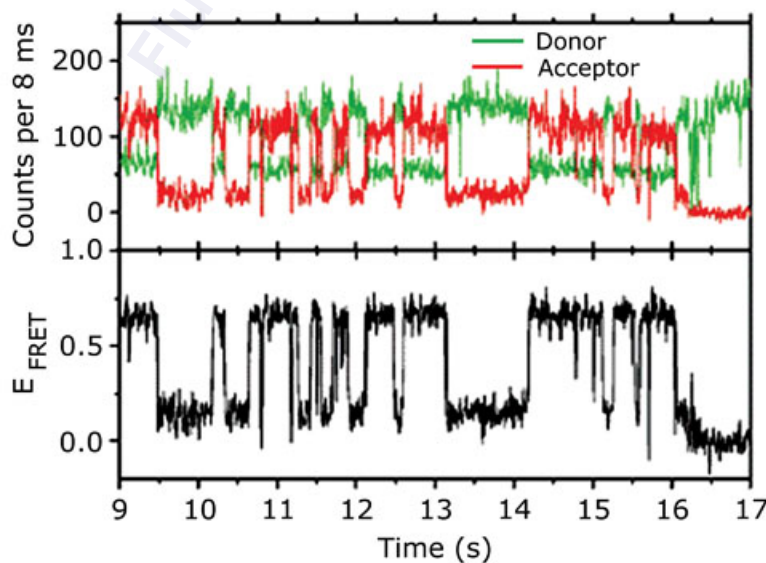
Junction 7

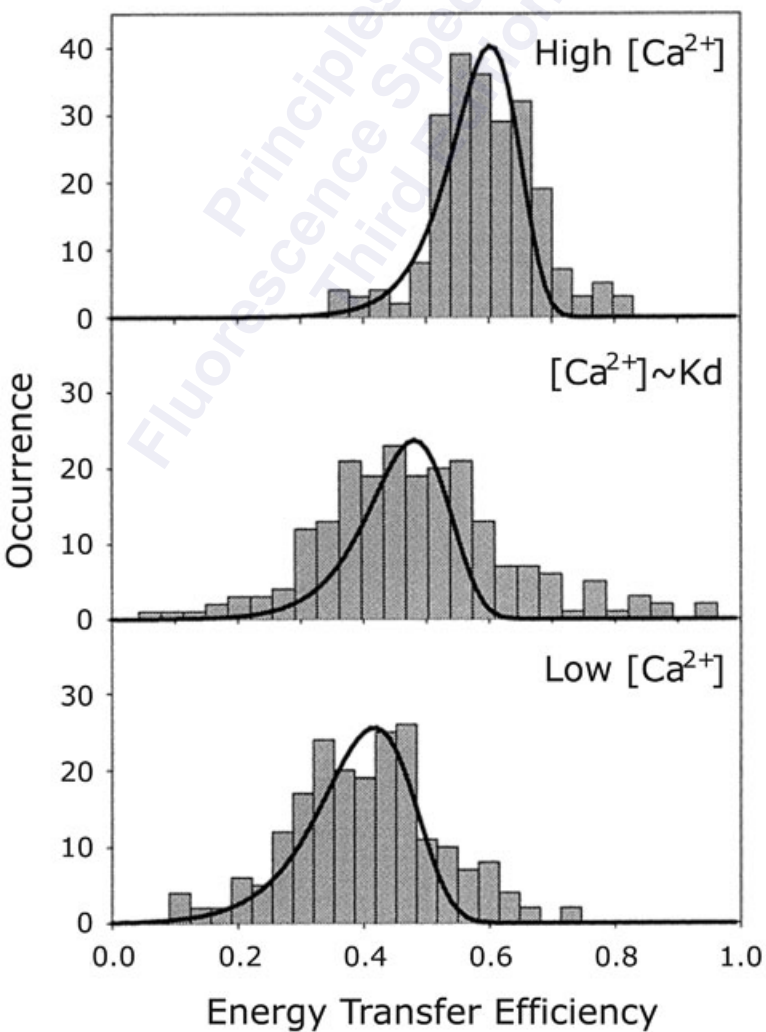
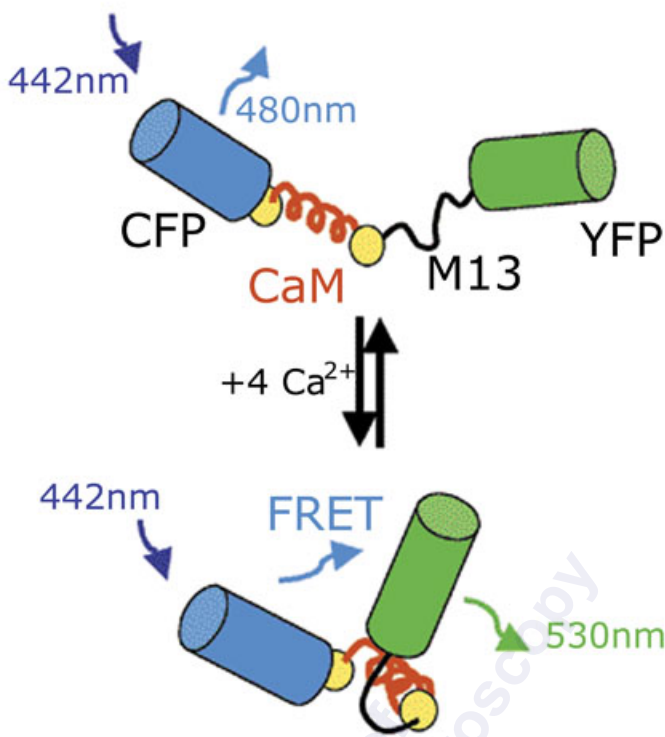


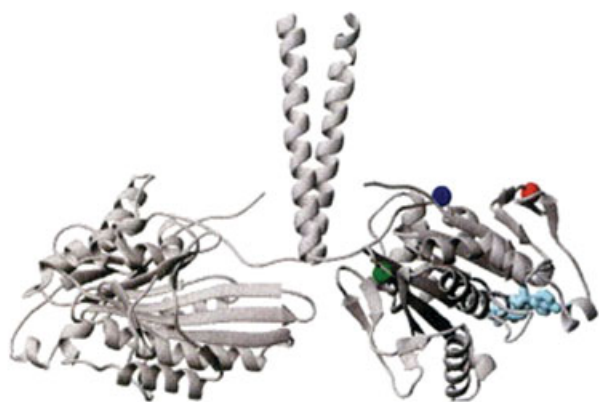
Junction 3



Junction 7

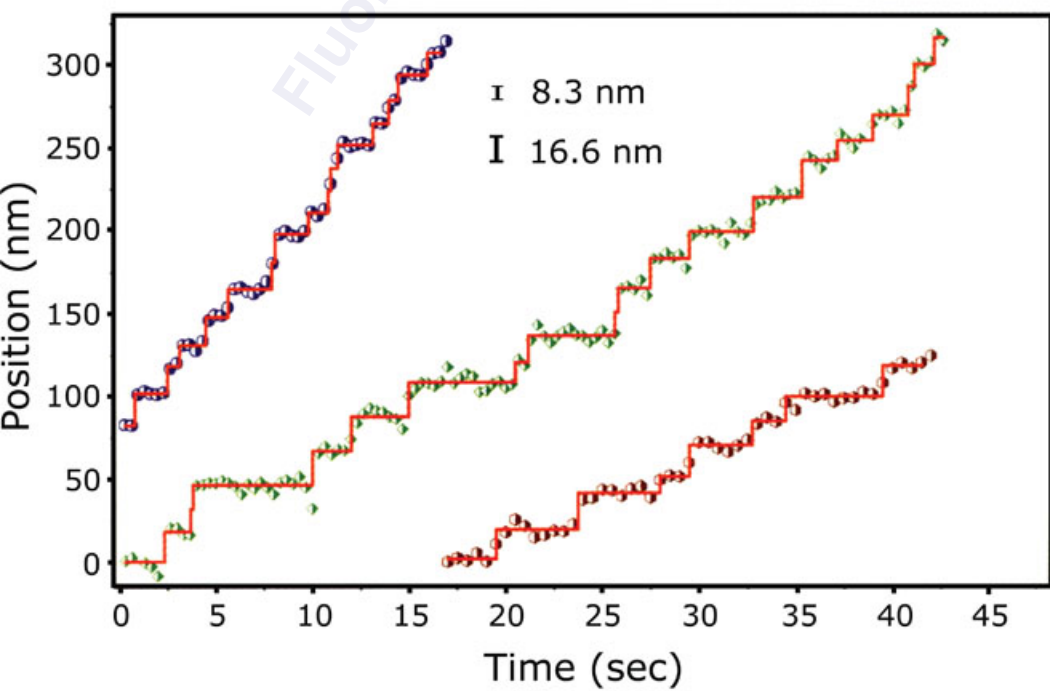
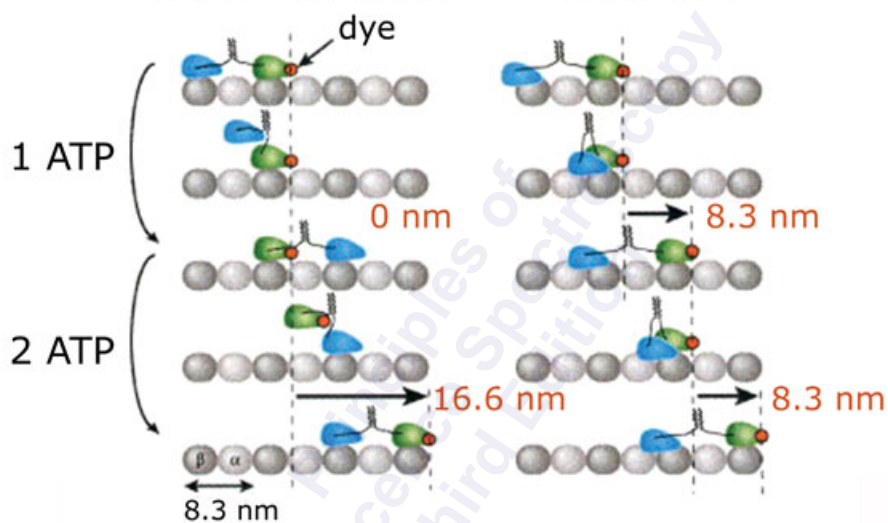


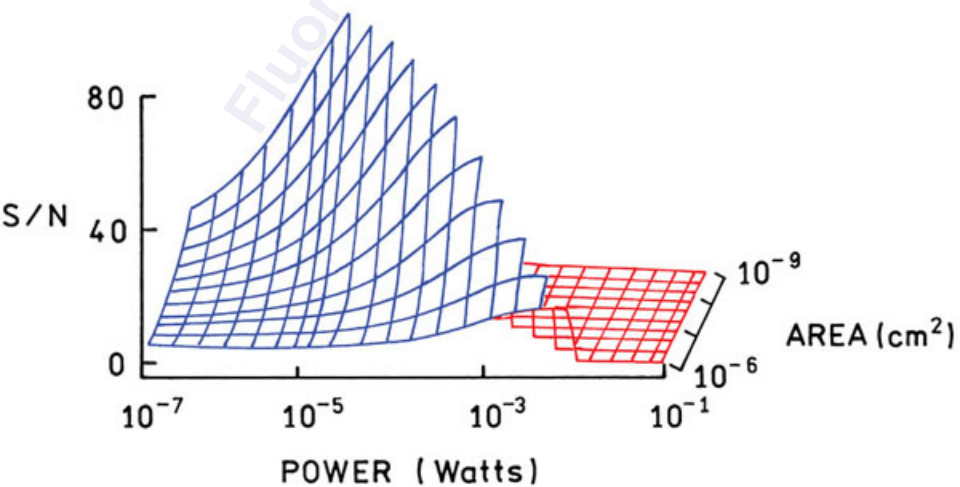
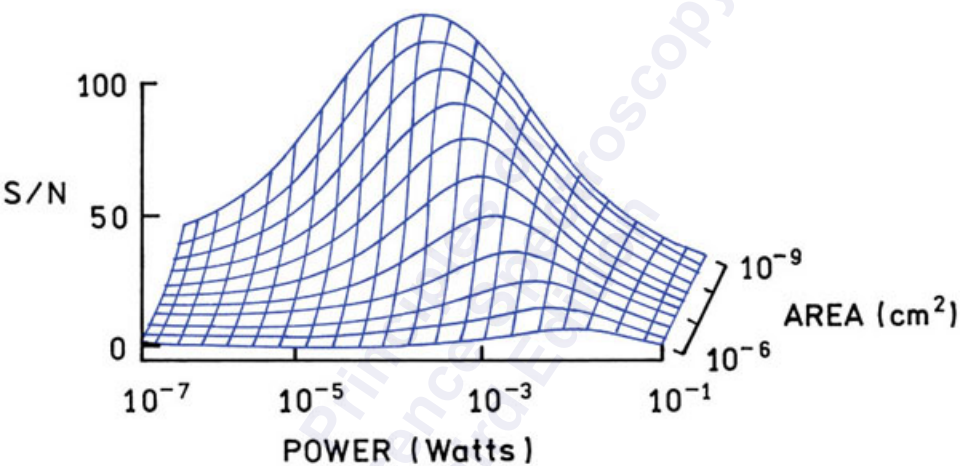
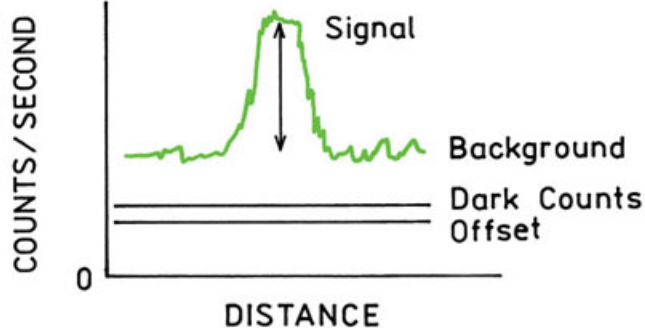


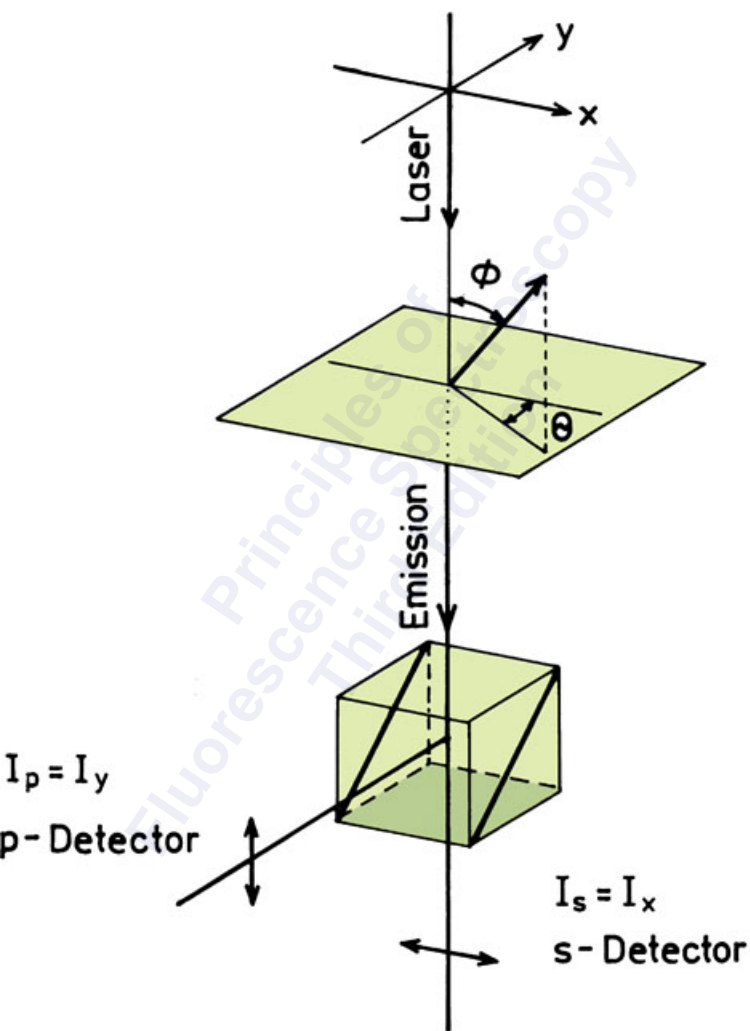


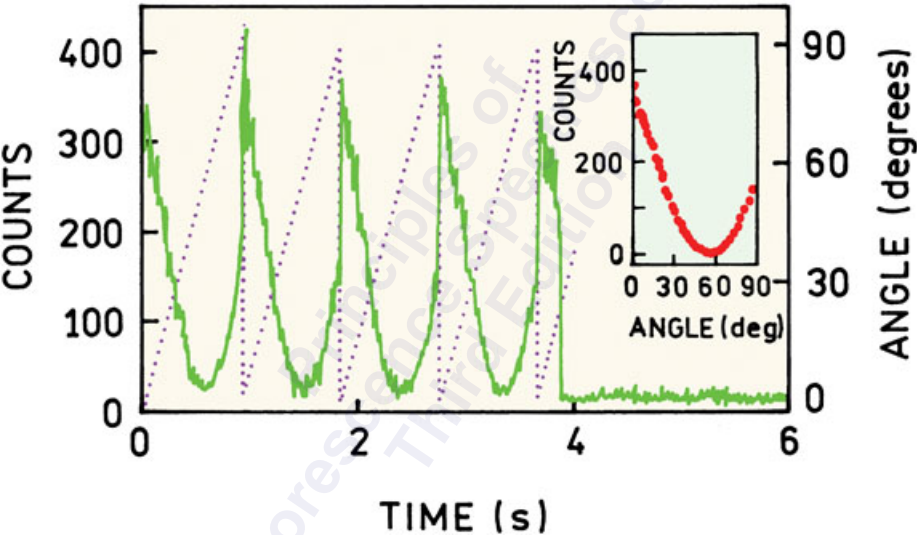
Hand-over-hand

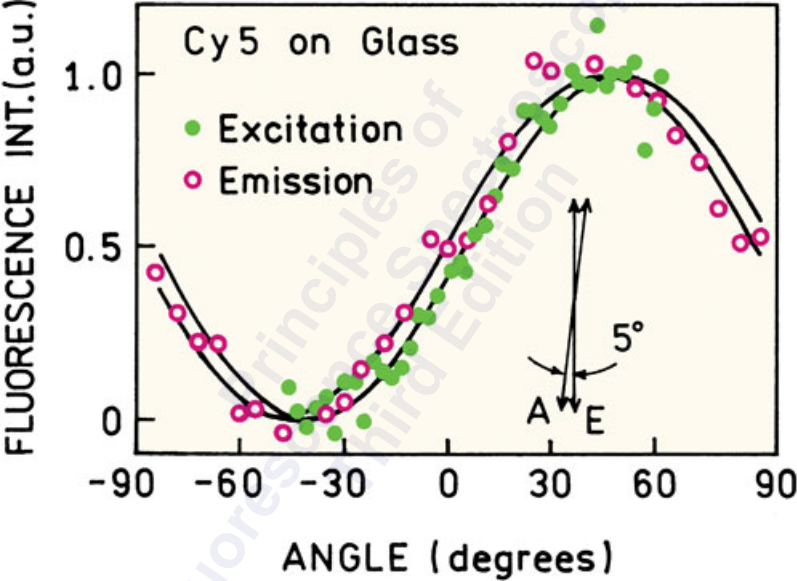
Inchworm



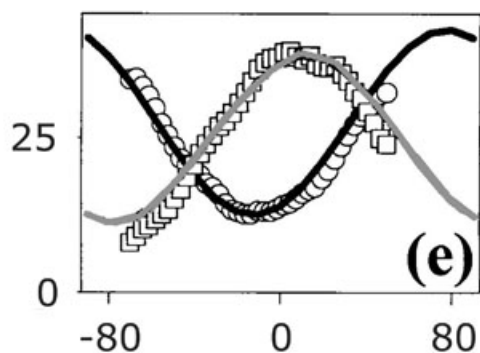
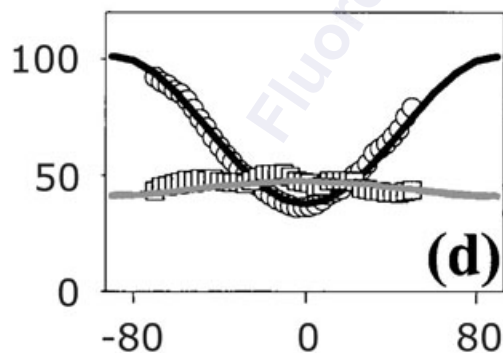
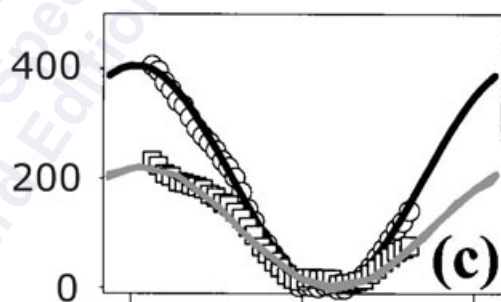
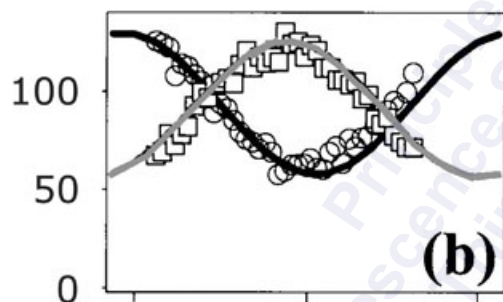
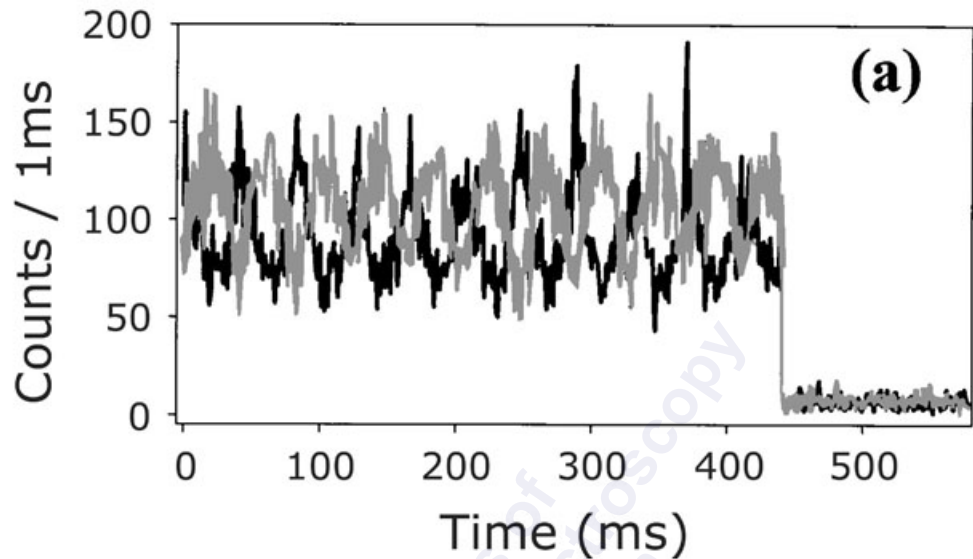




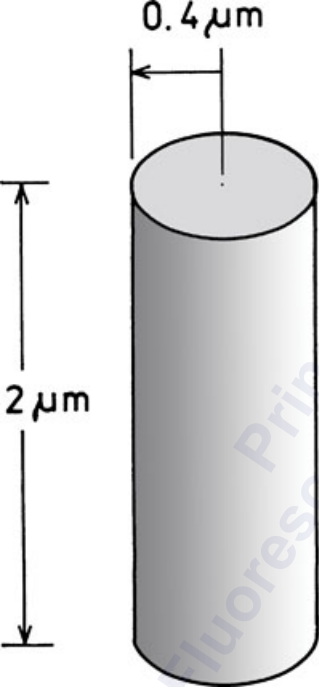








Angle (degrees)



Volume = 1 fl

$c = 1\ \text{nM}$

$N = 0.6\ \text{molecules}$

$$P(0) = 0.55$$

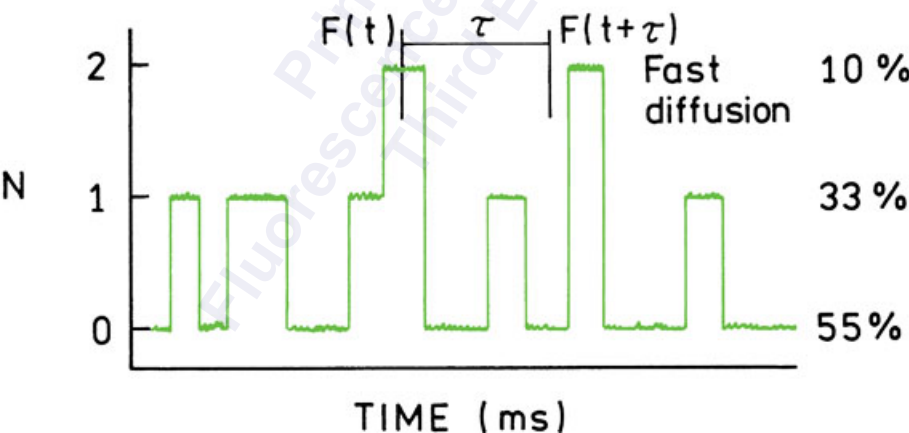
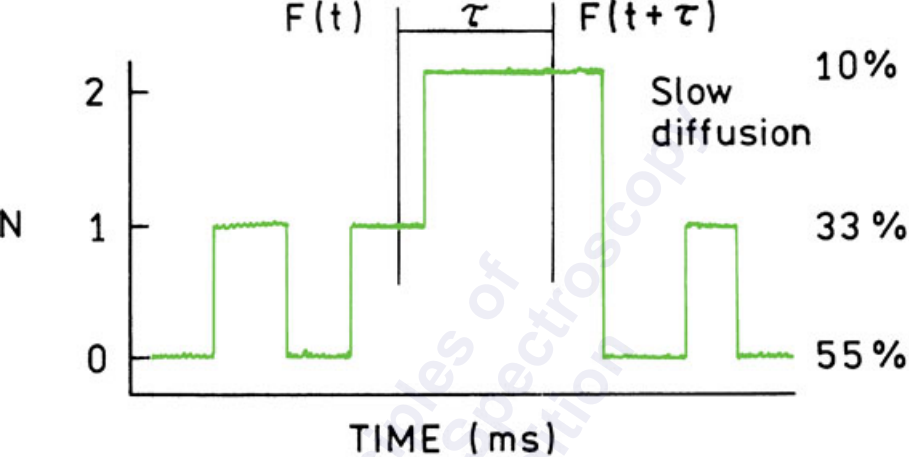


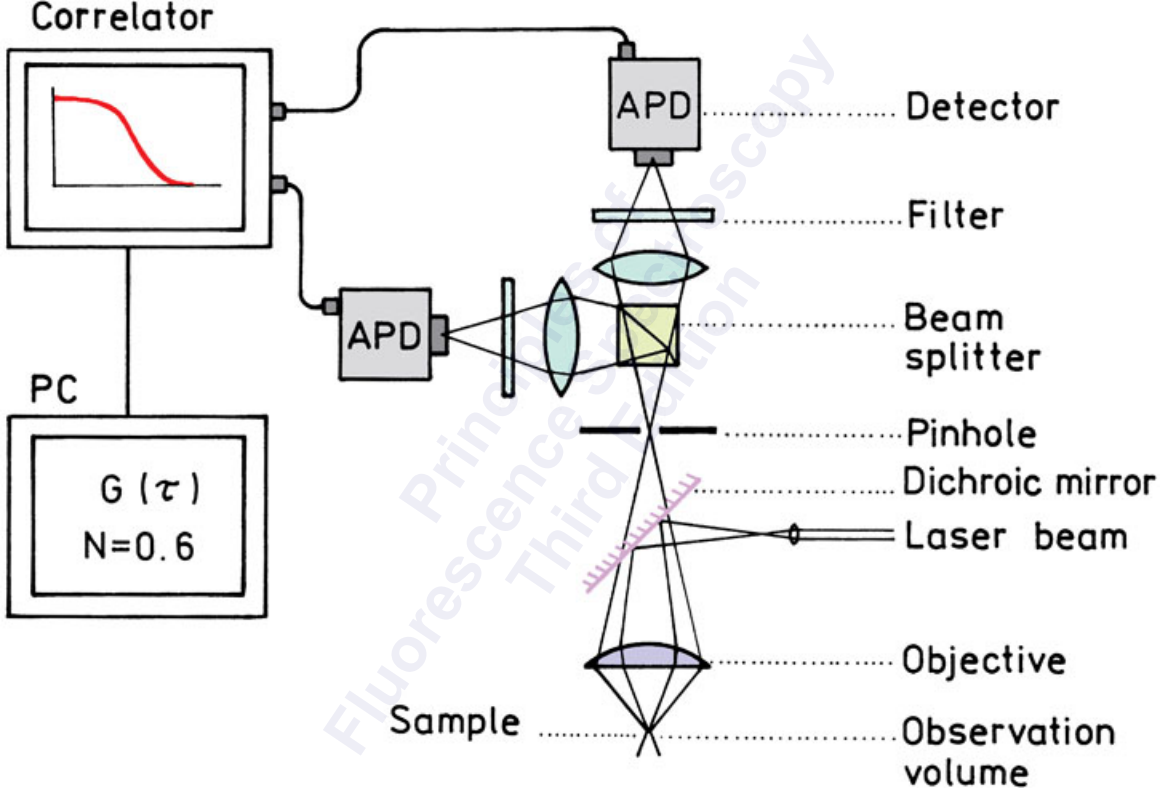
$$P(1) = 0.33$$

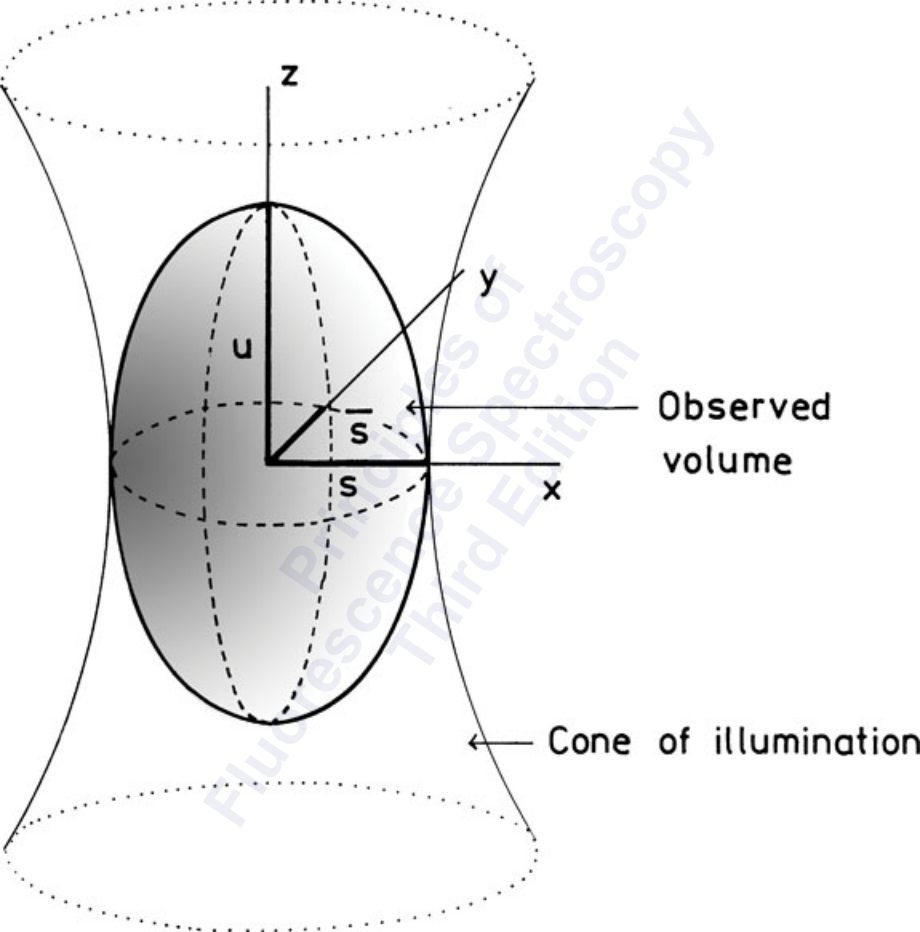


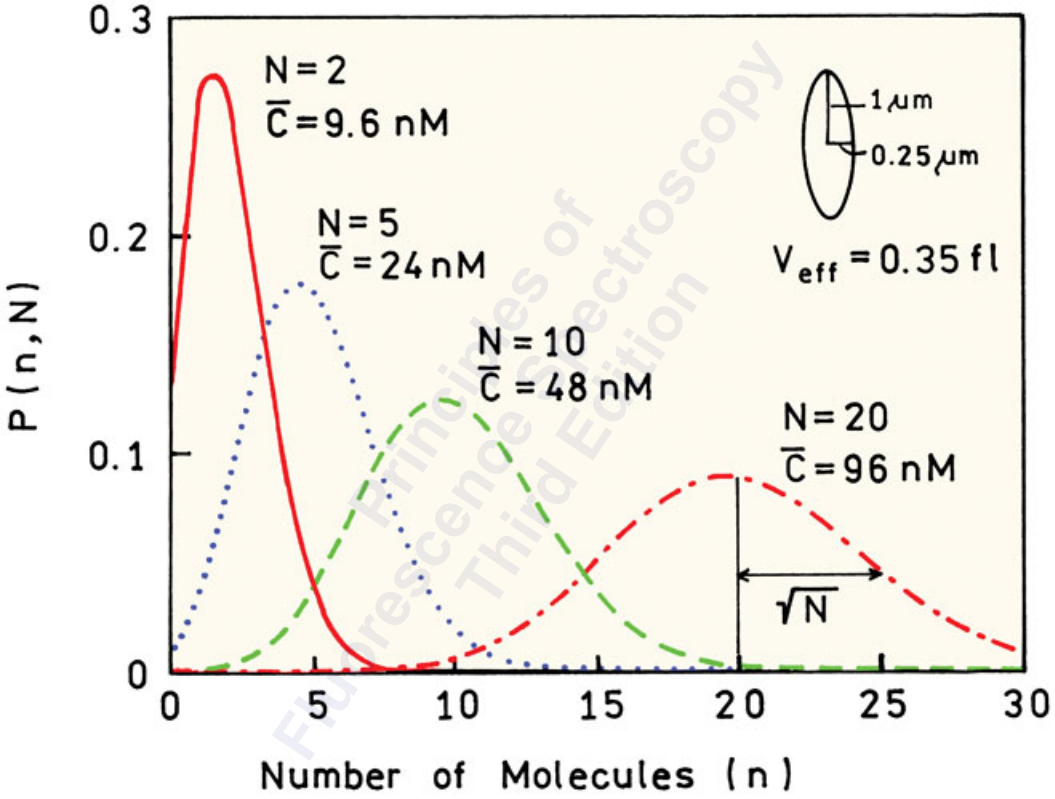
$$P(2) = 0.10$$

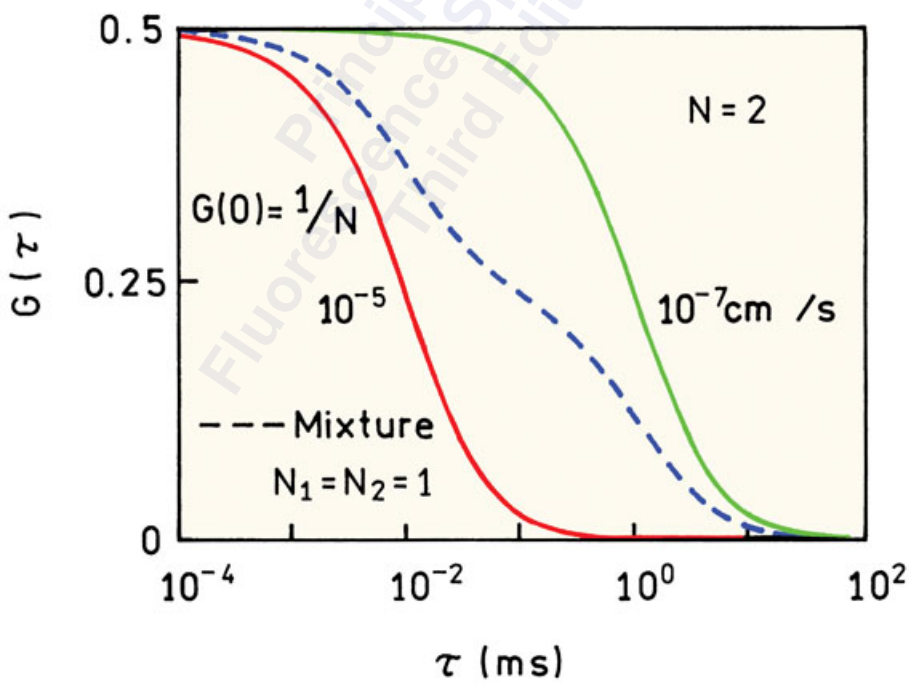
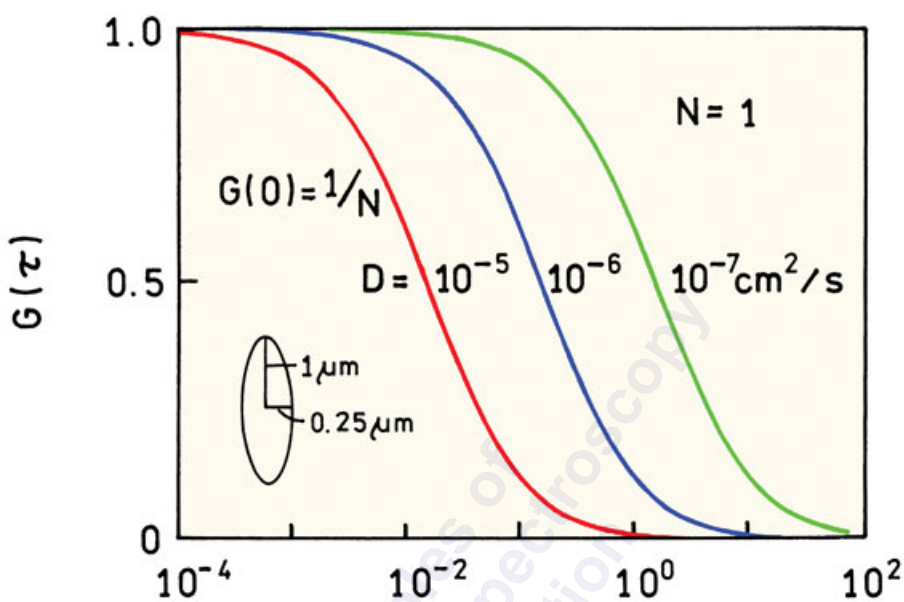




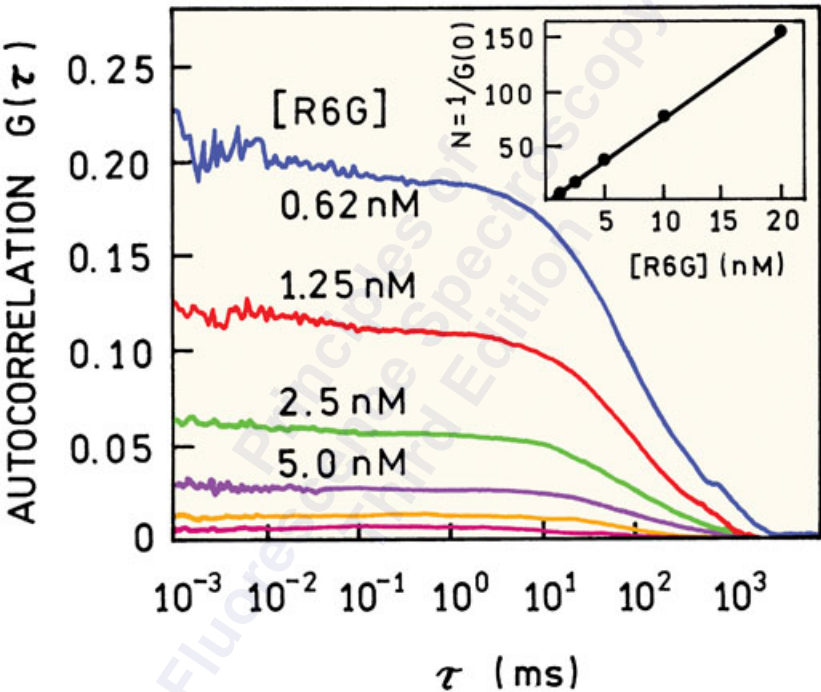




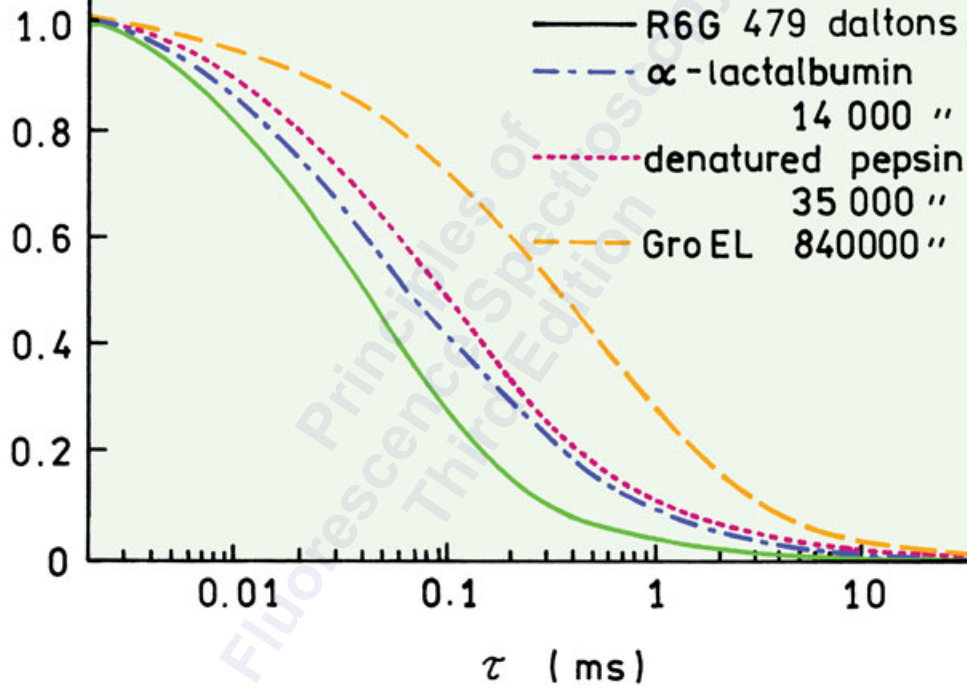


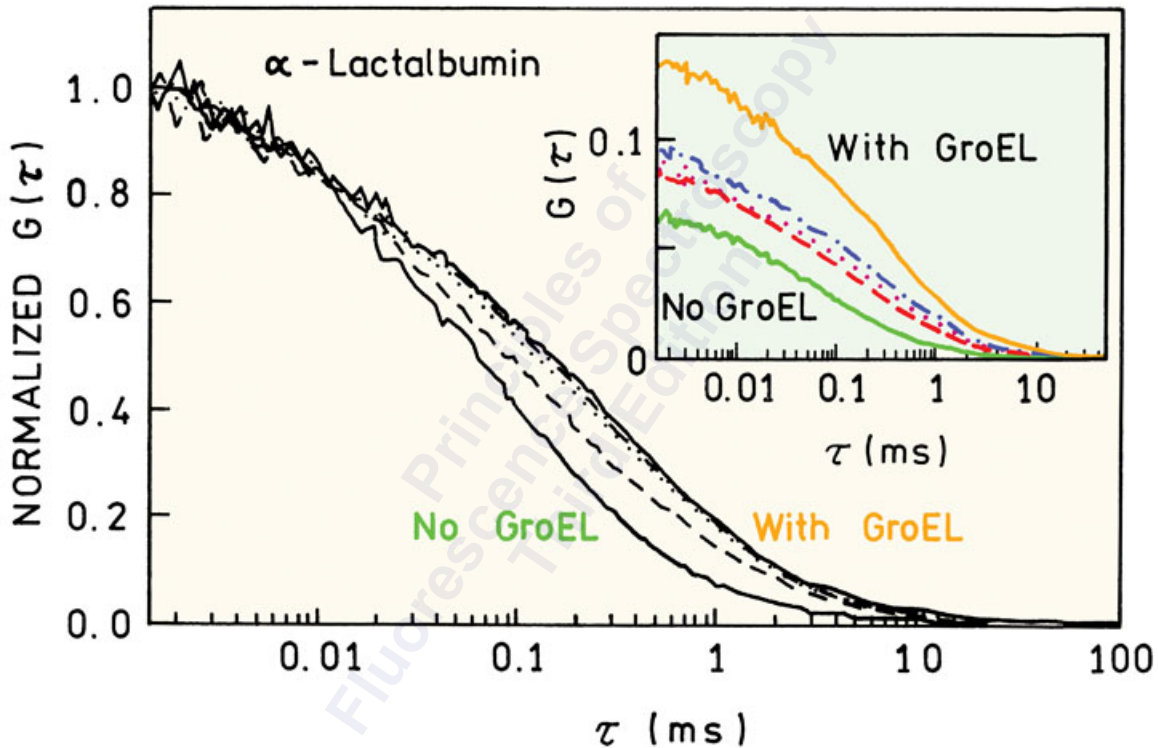


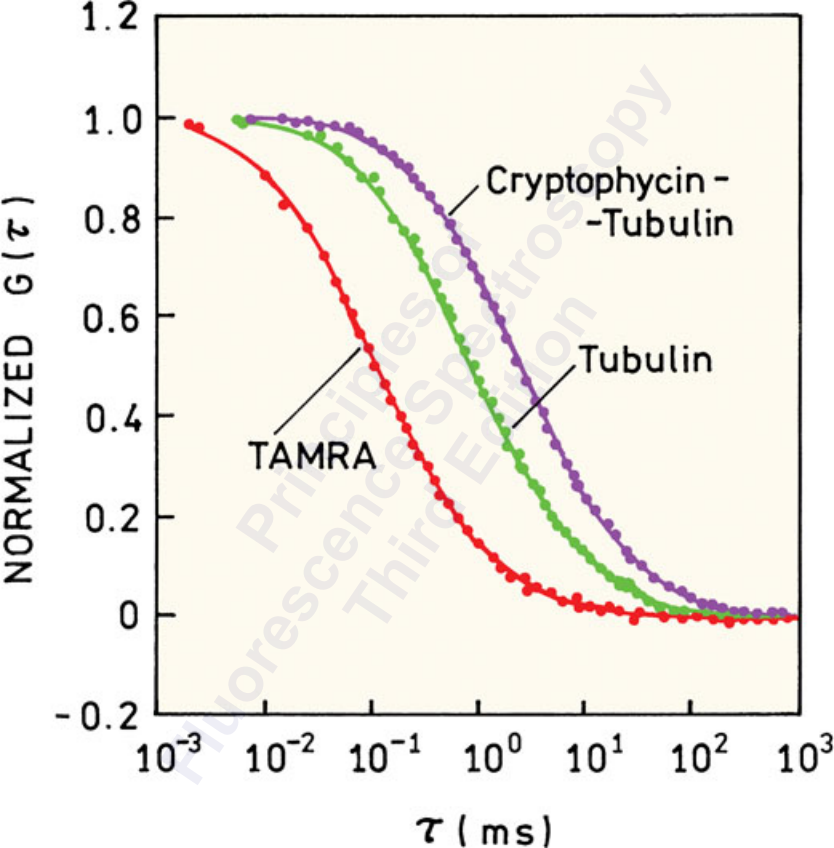




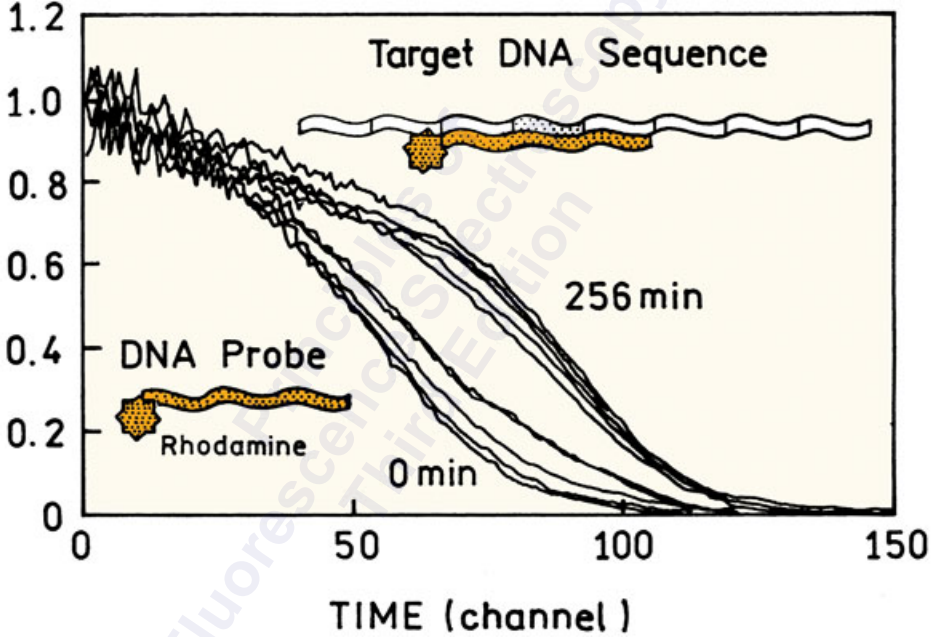
NORMALIZED  $G(\tau)$

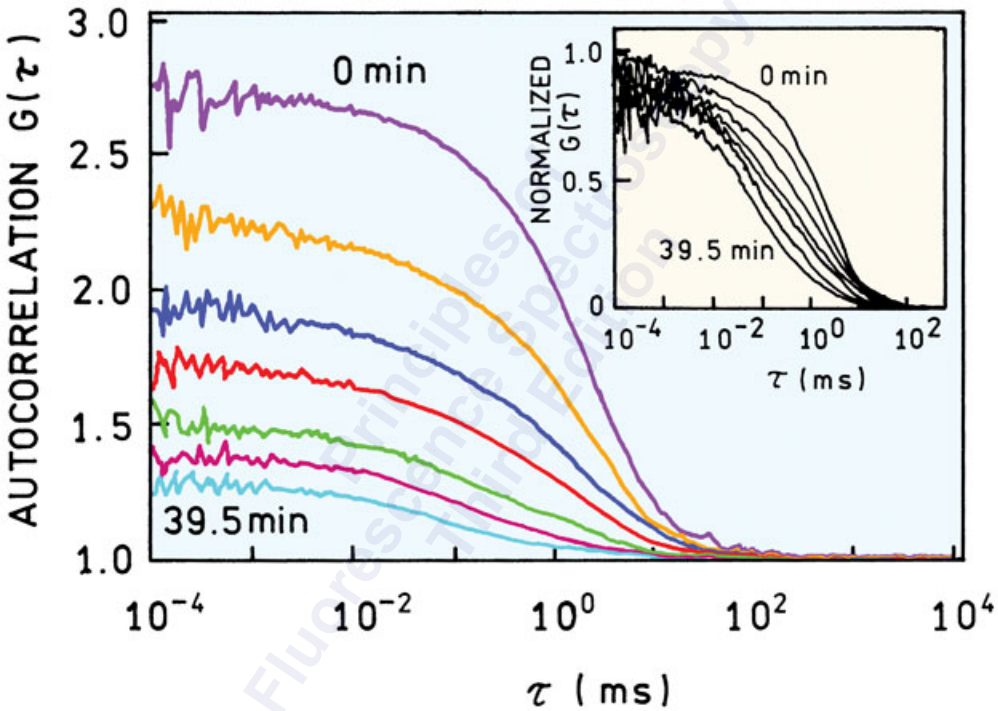


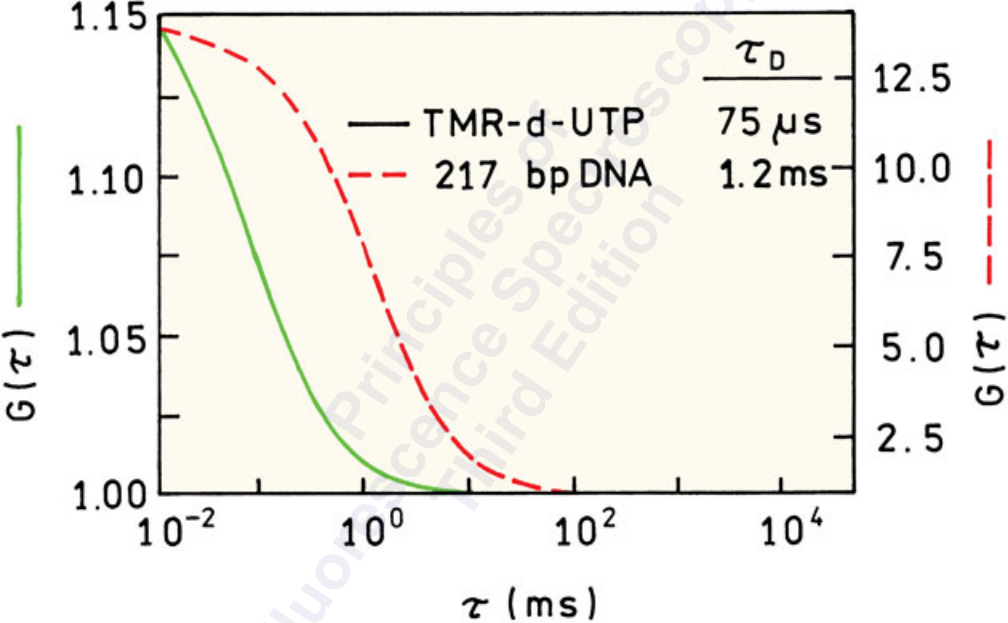


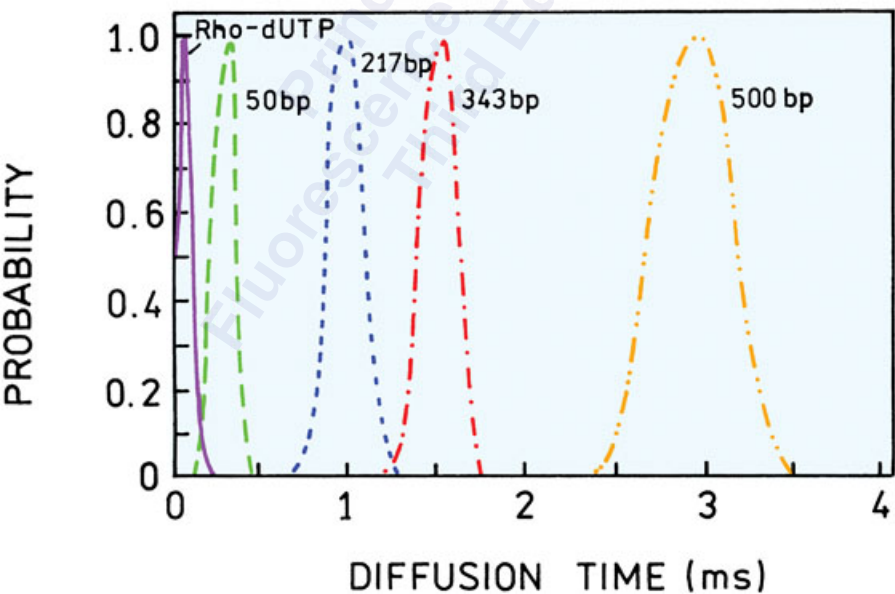
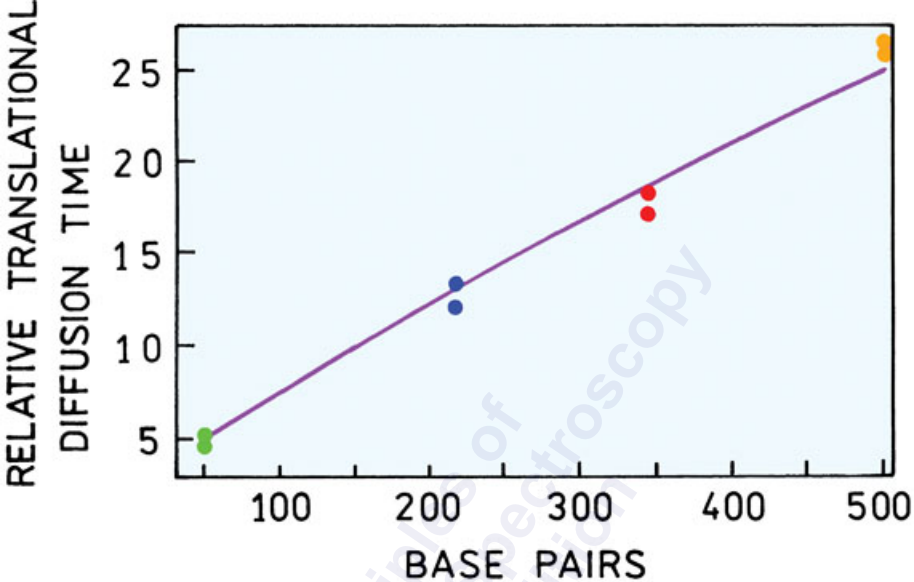


AUTOCORRELATION  
FUNCTION (a.u.)

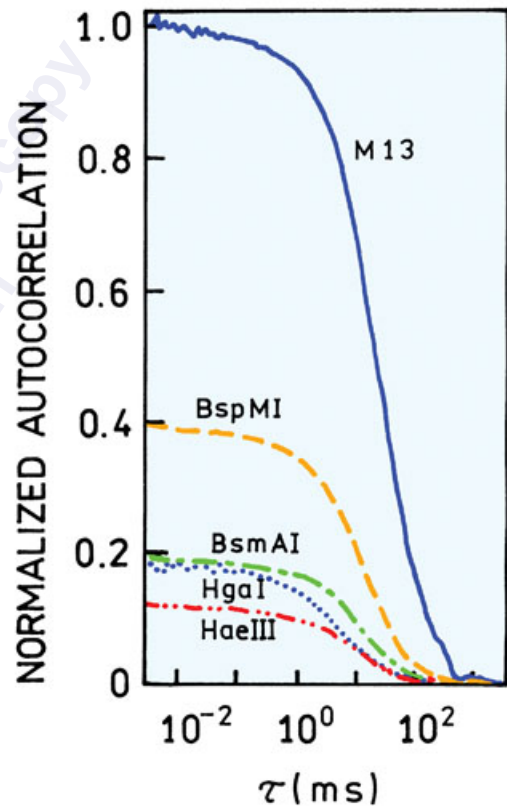
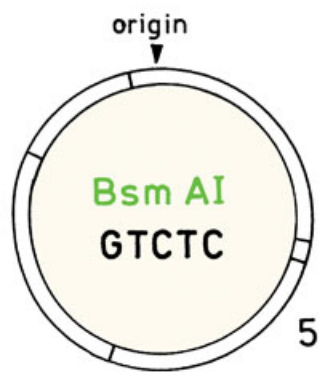
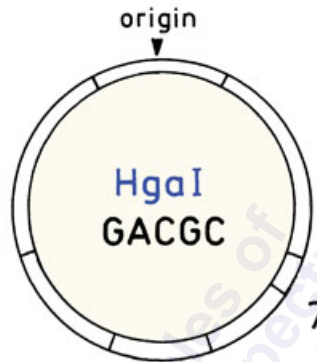
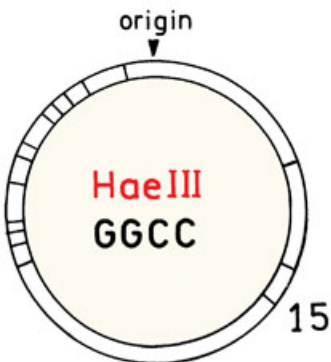


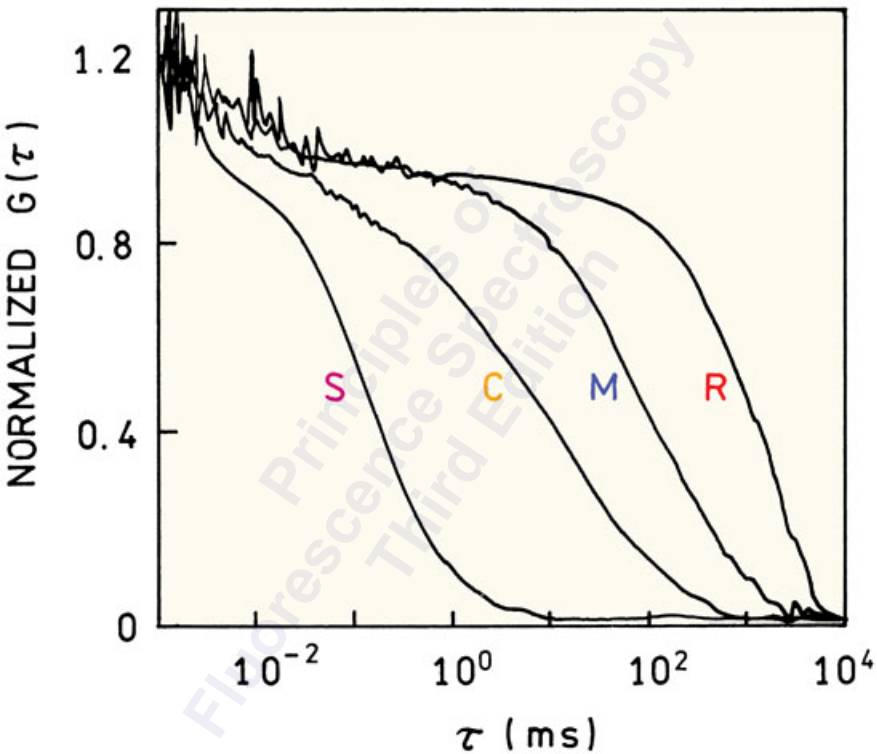


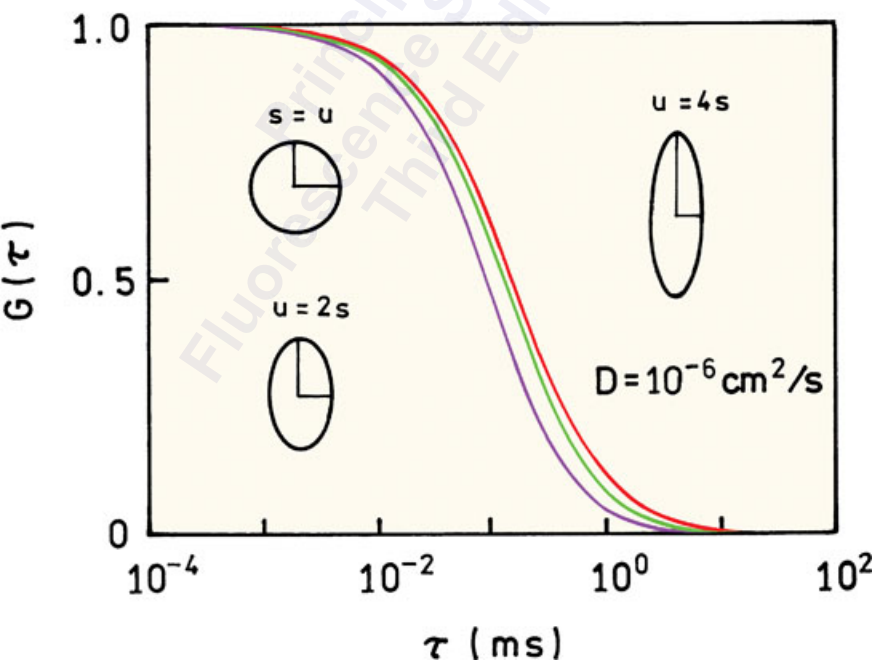
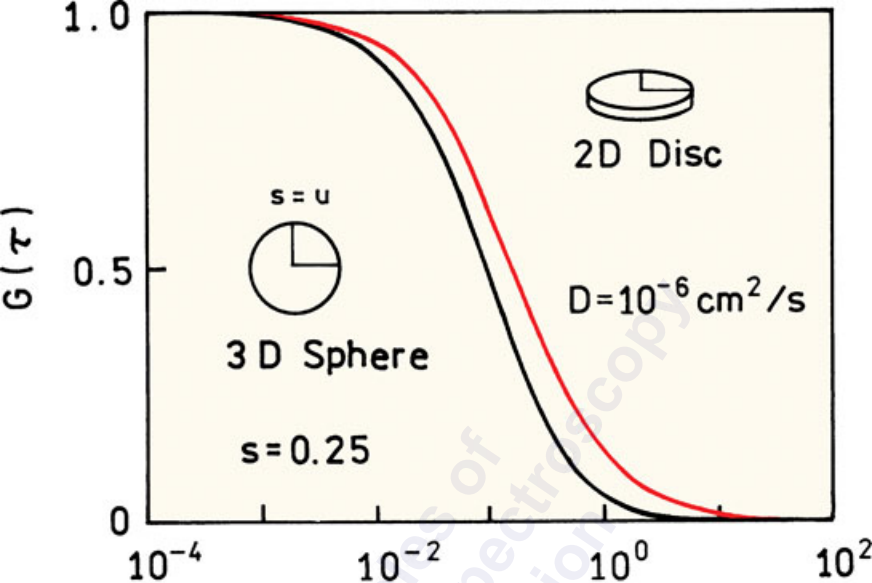


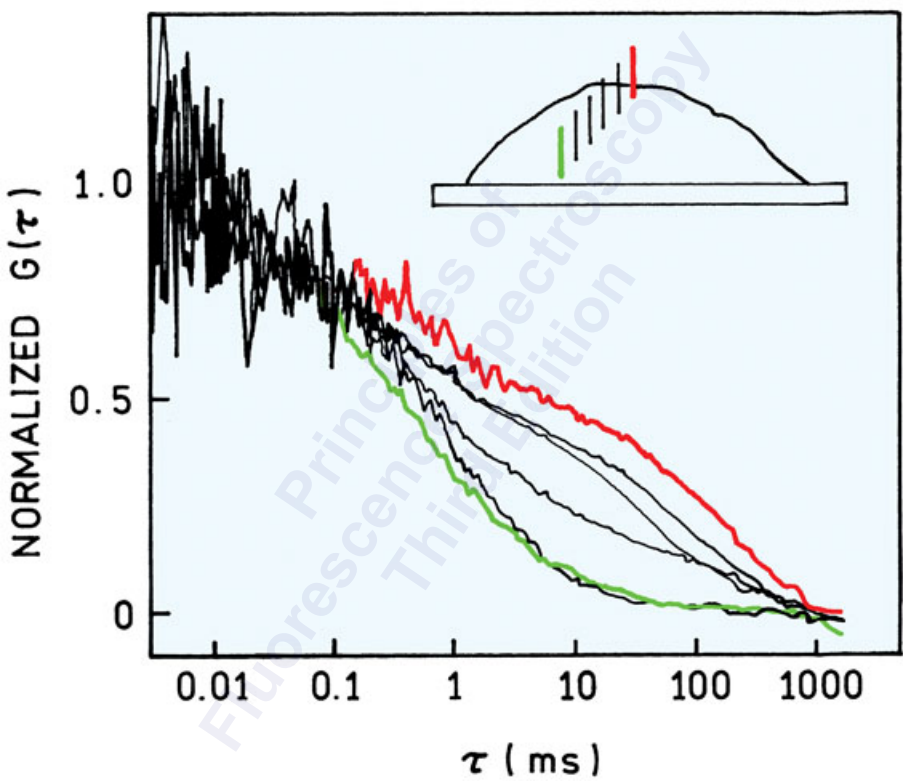


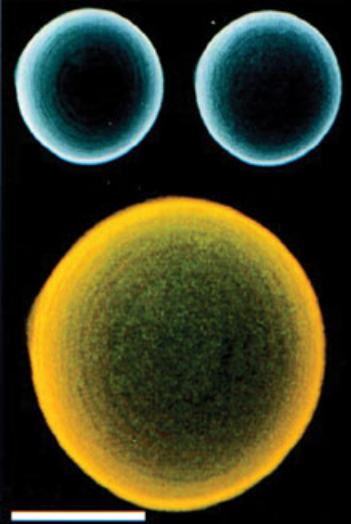




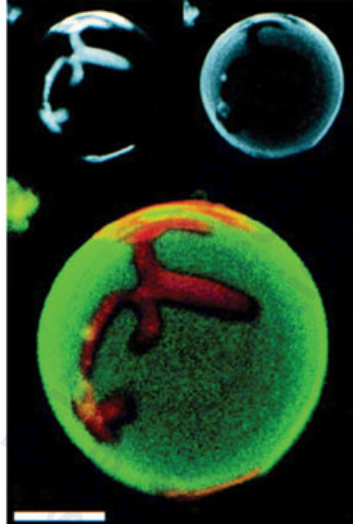




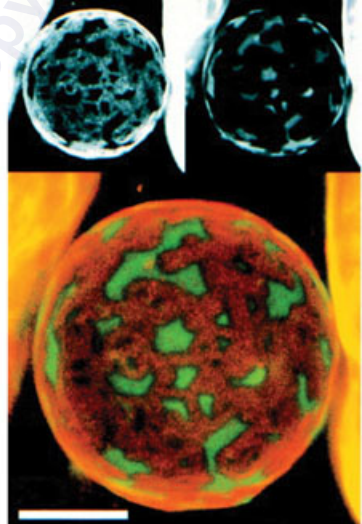




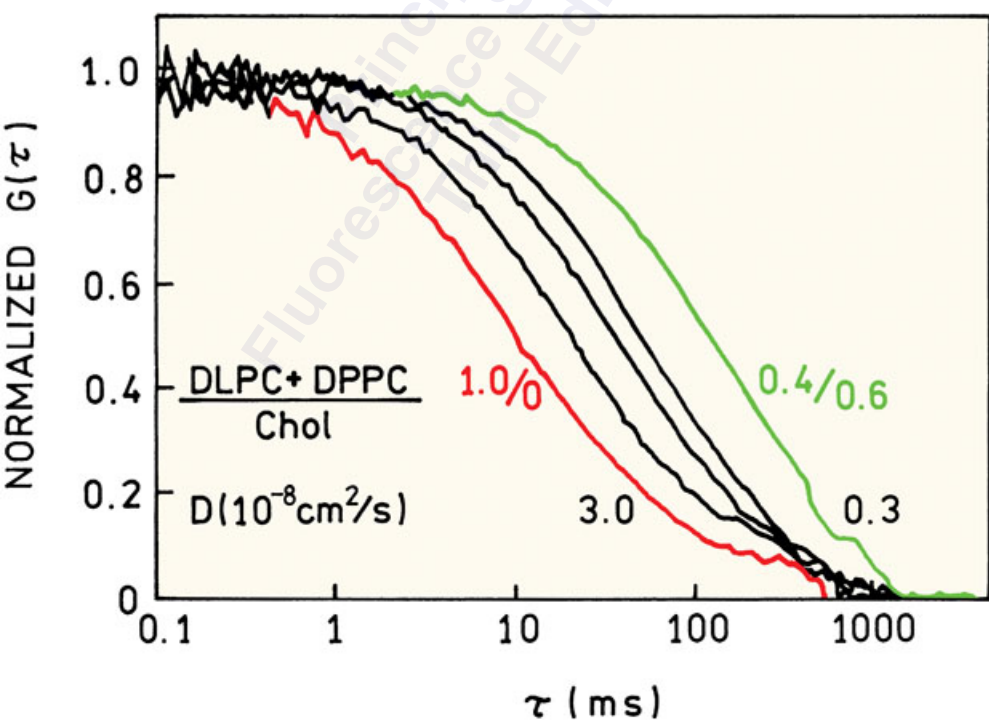
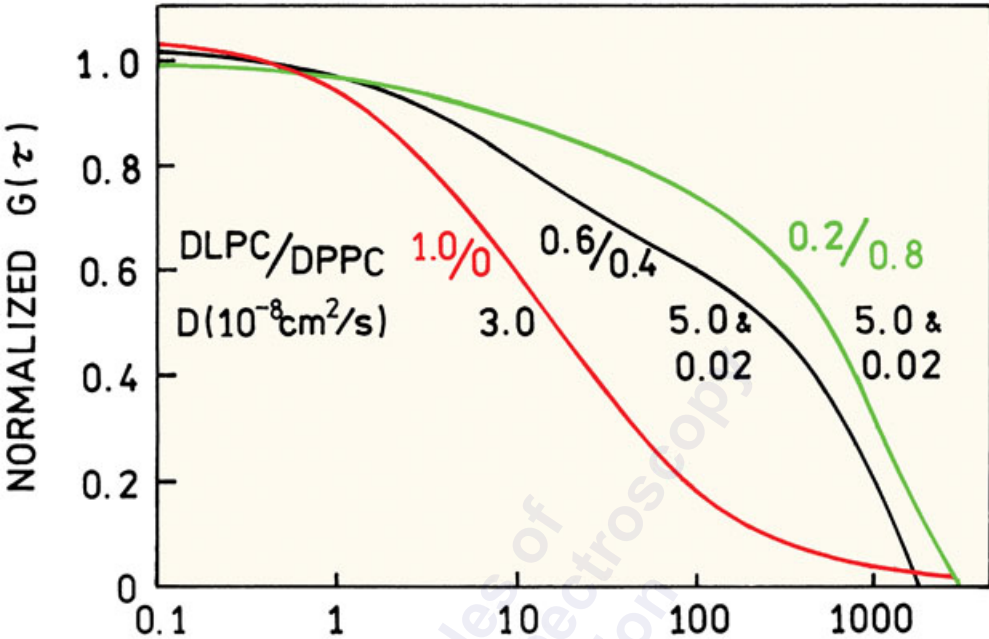
1.0 / 0.0

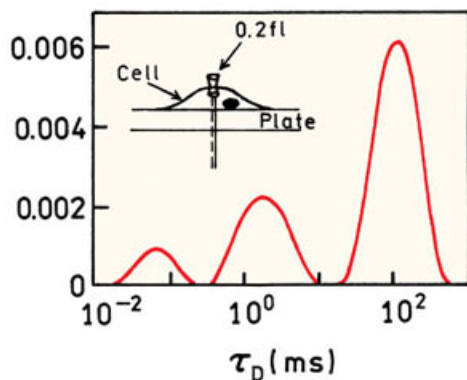
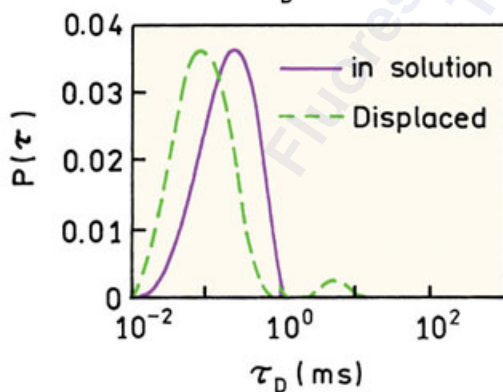
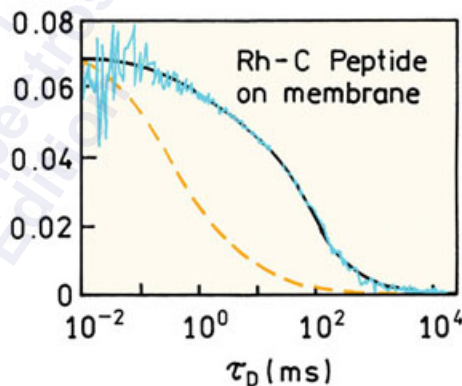
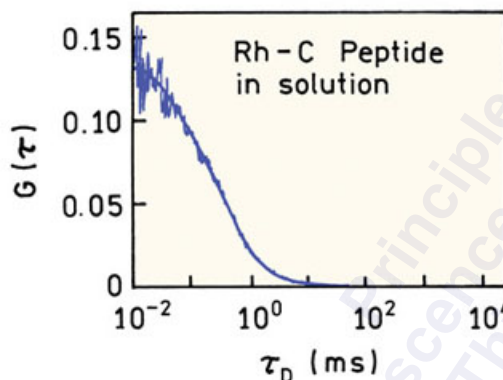
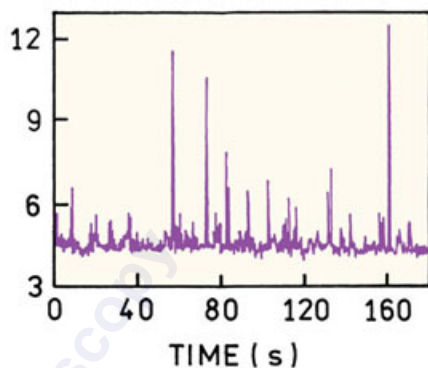
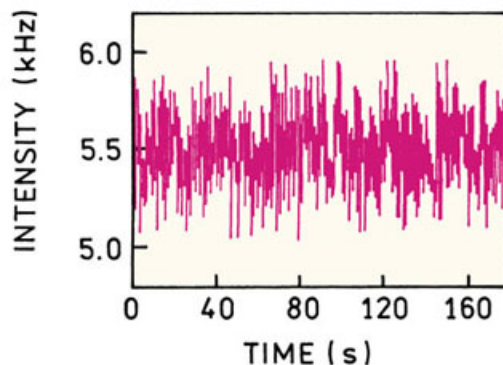


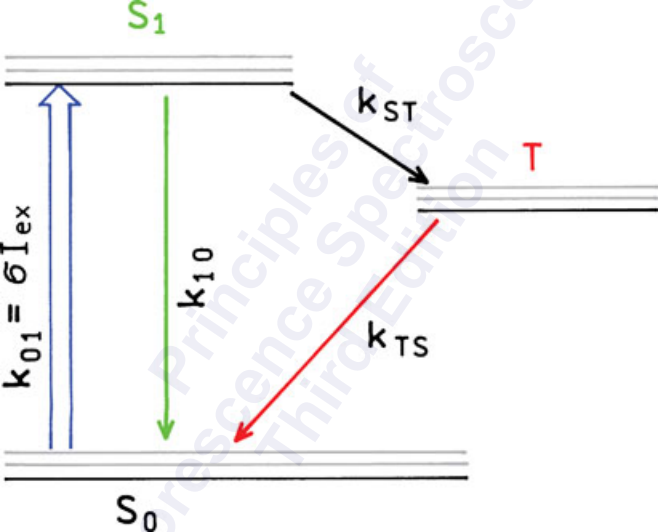
0.6 / 0.4  
DLPC / DPPC



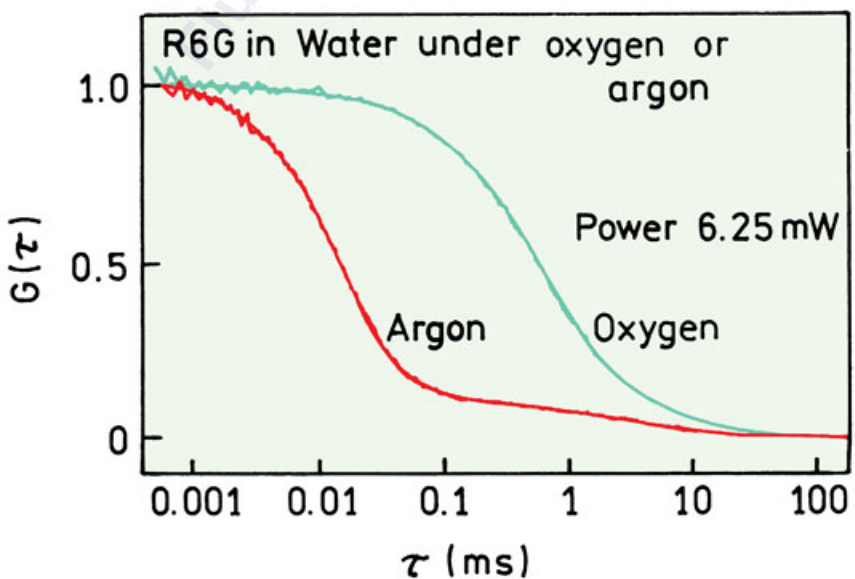
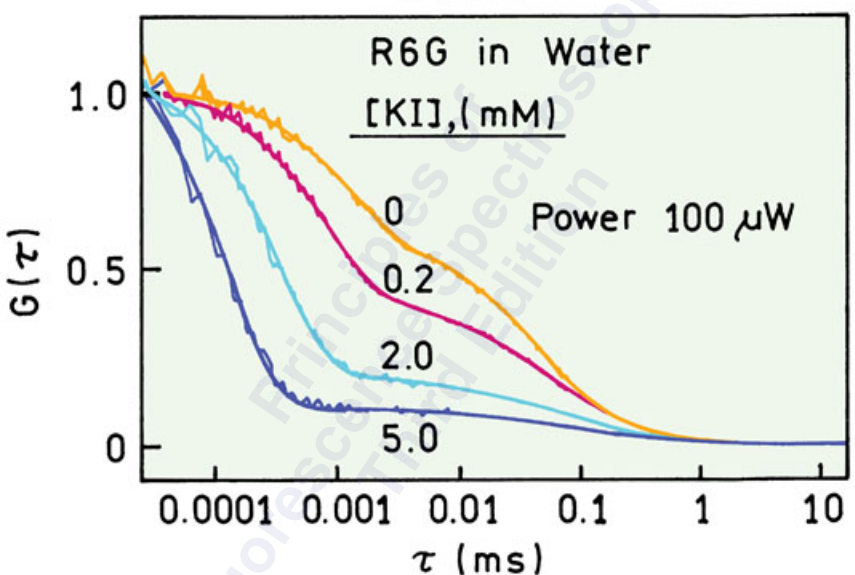
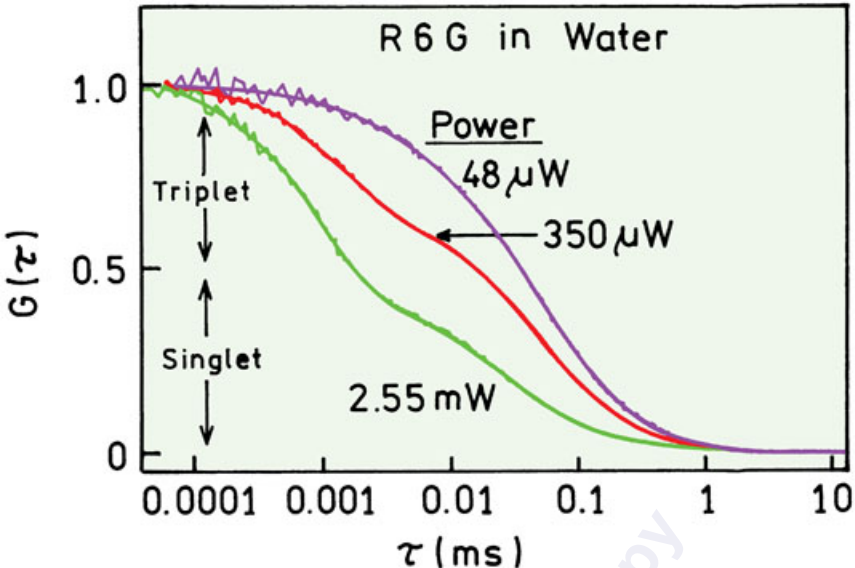
0.2 / 0.8

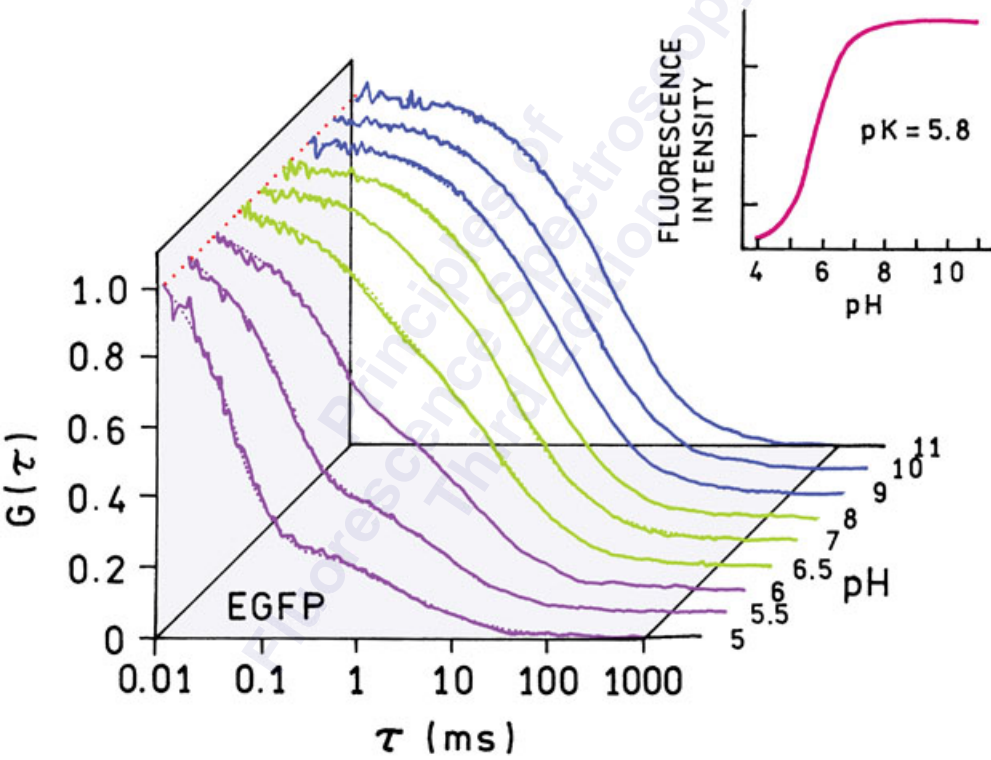




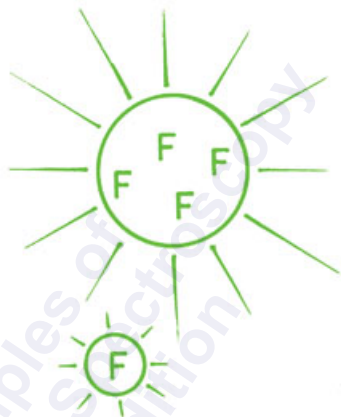




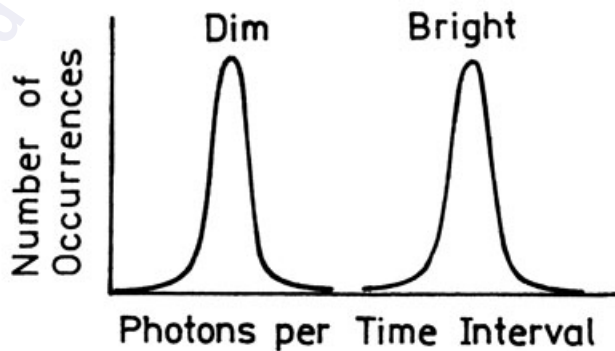
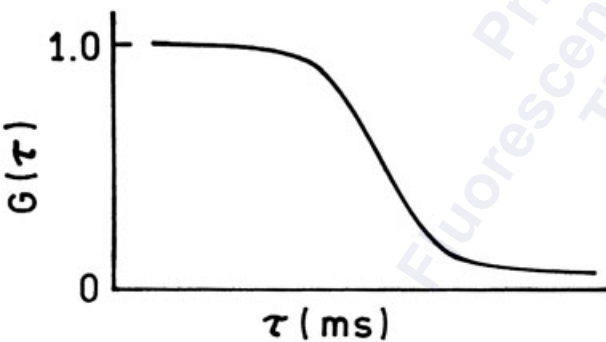


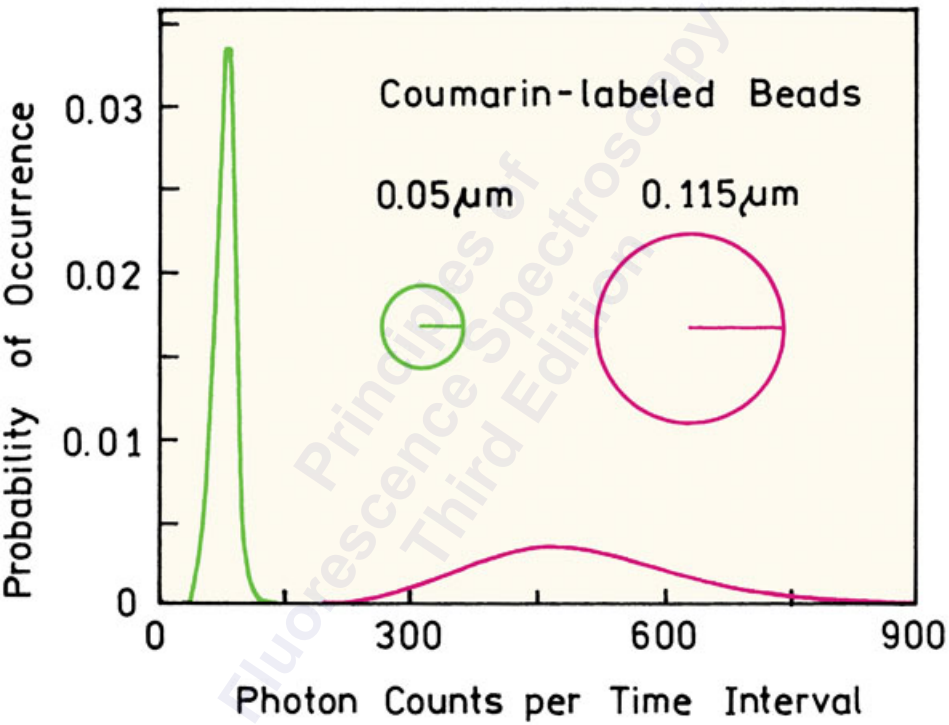


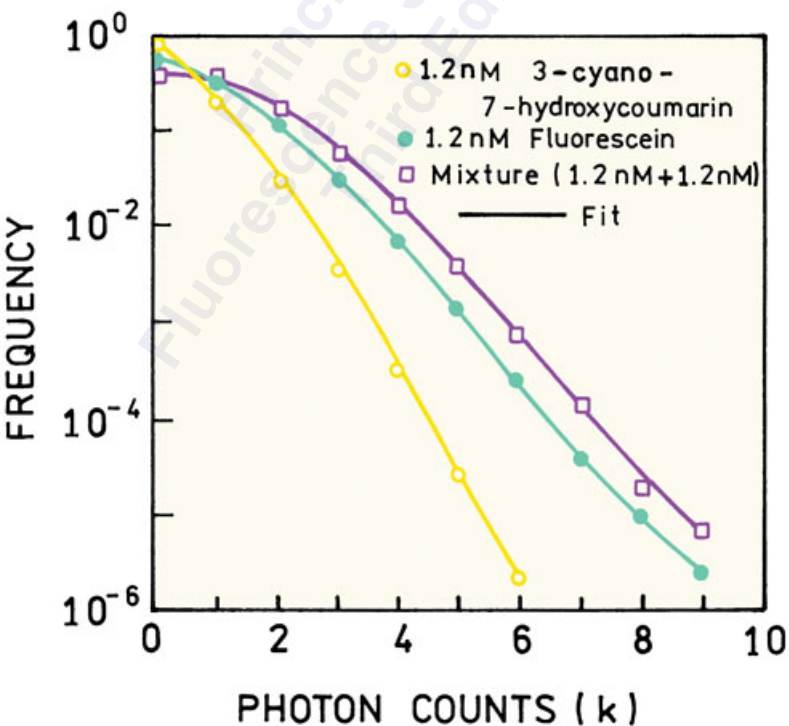
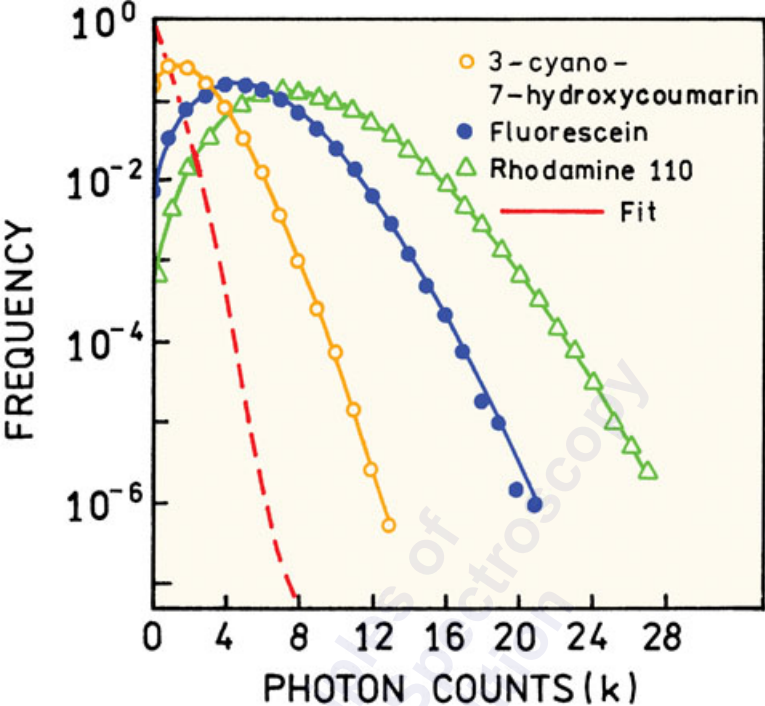
Dim

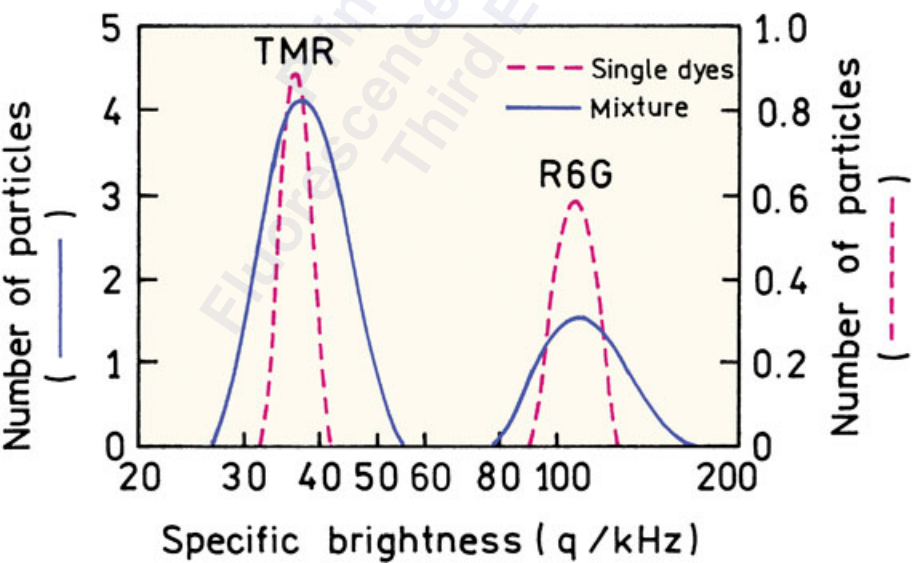
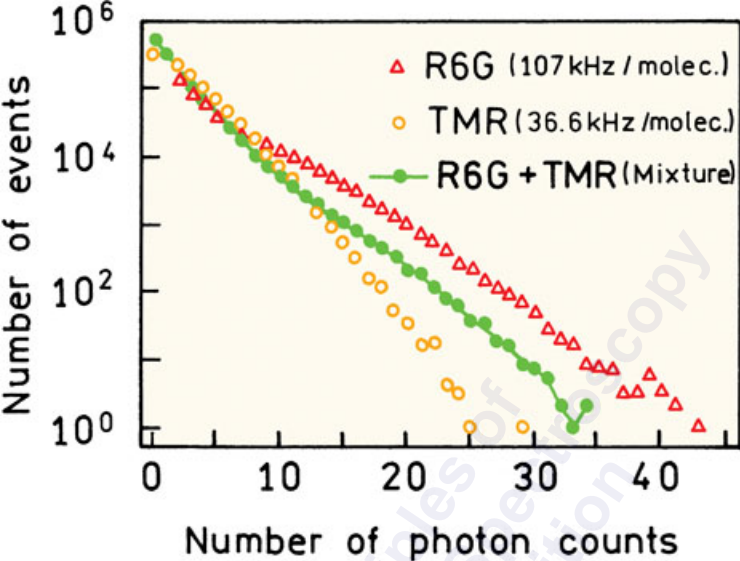


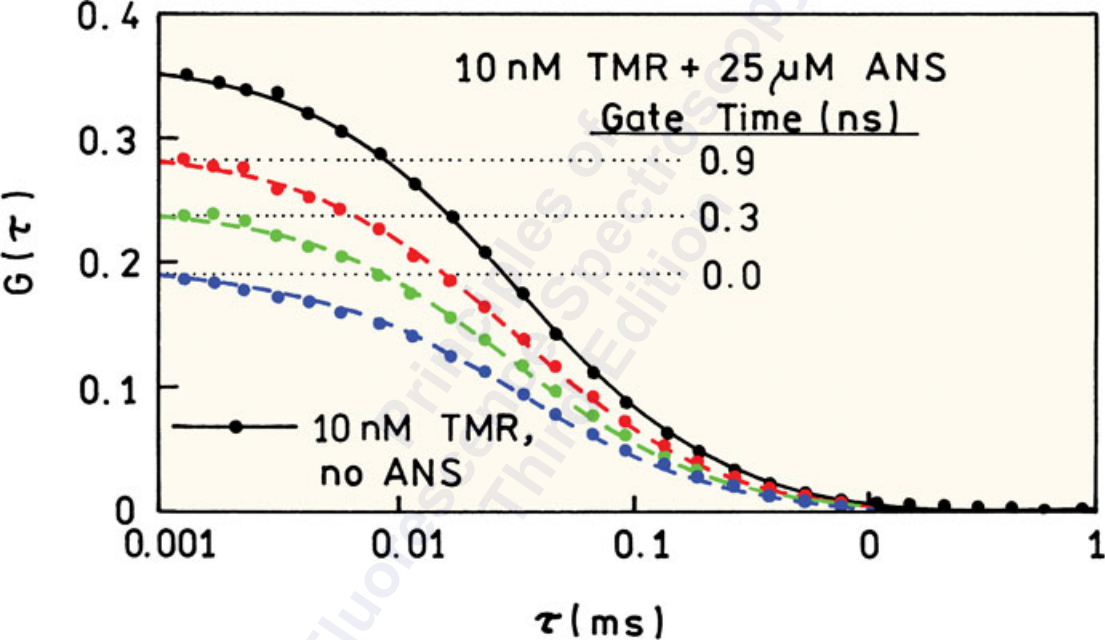
Bright

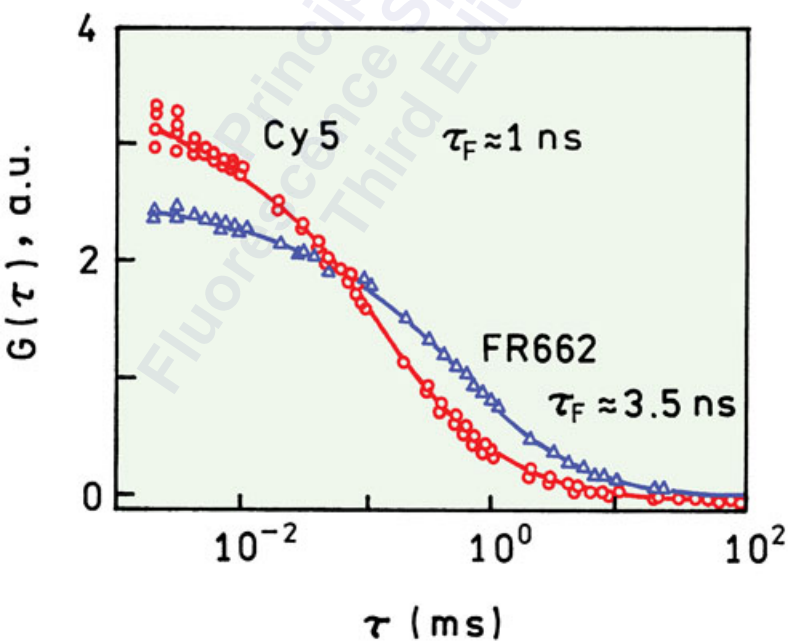
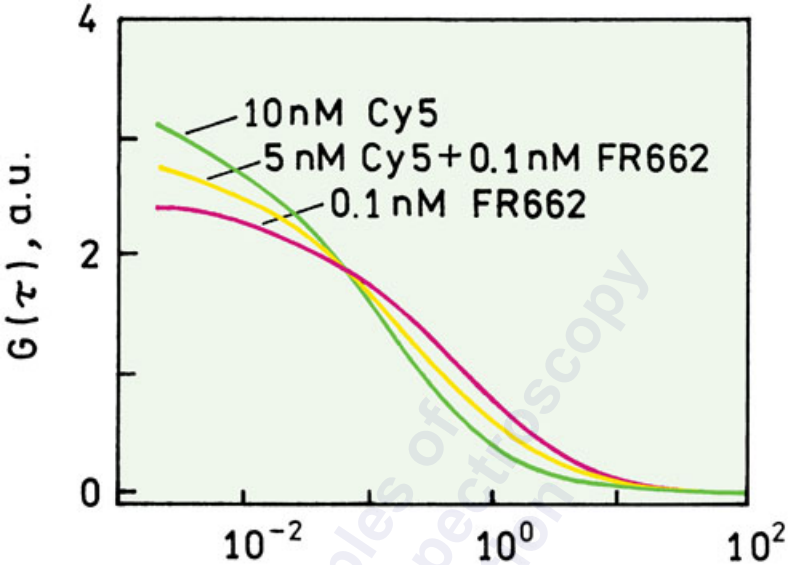




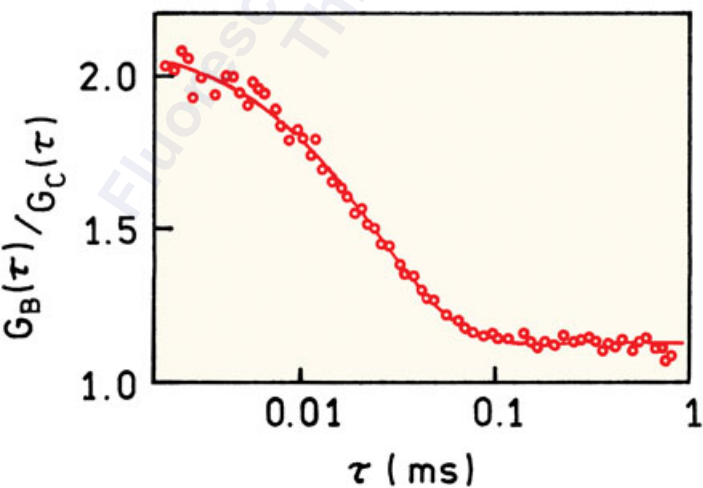
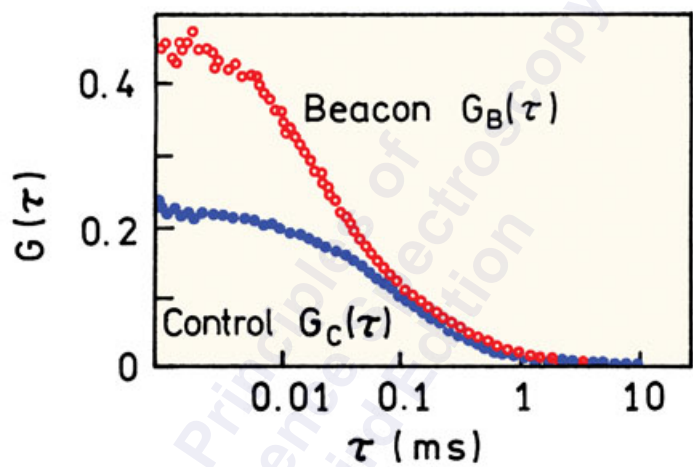
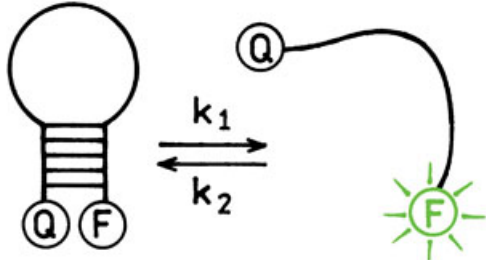


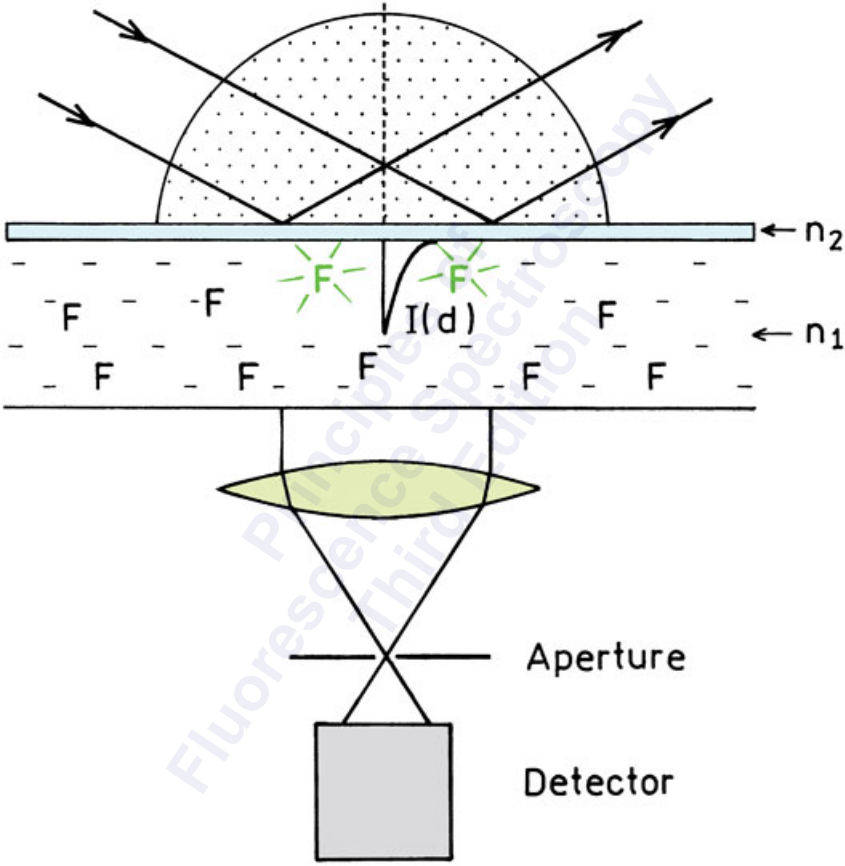


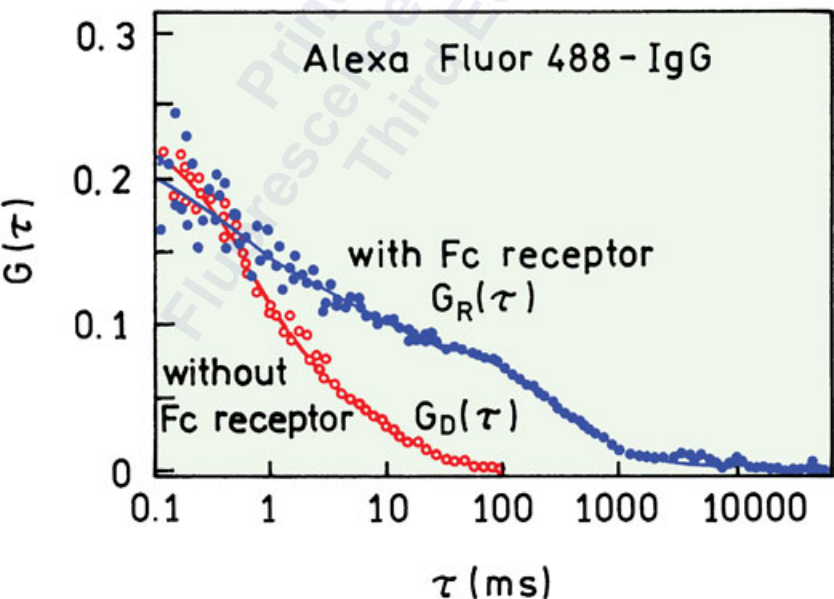
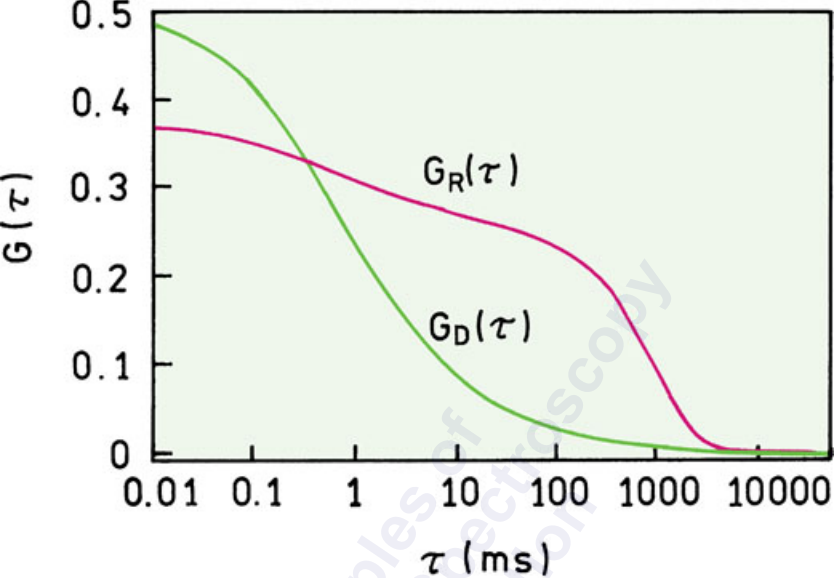




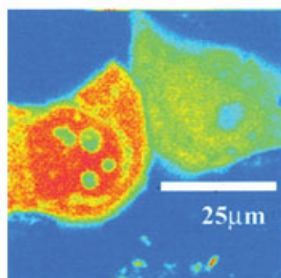




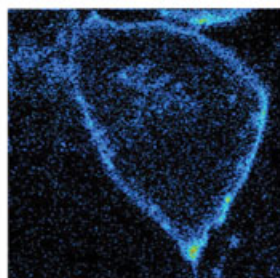




AKC-EGFP



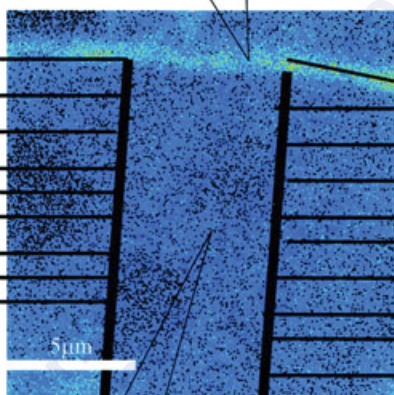
AKM-EGFP



Plasma Membrane

 $\mu\text{m}^2/\text{s}$ 

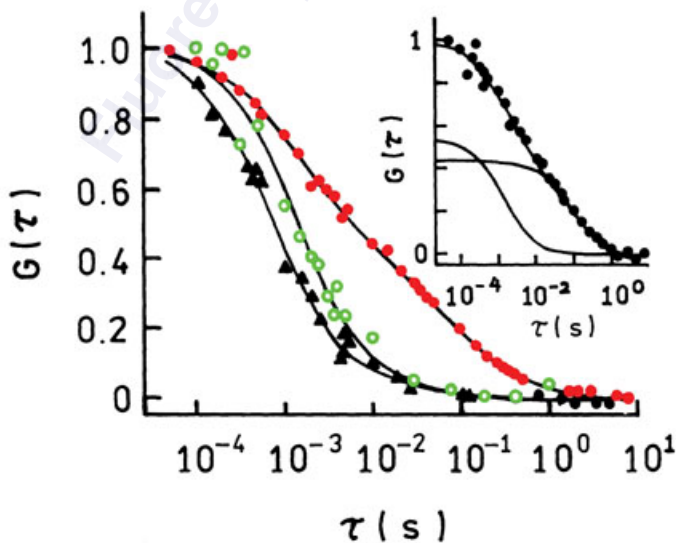
13/0.18
16
11.5
11.6
12.2
8.5
13.9
11.4
11

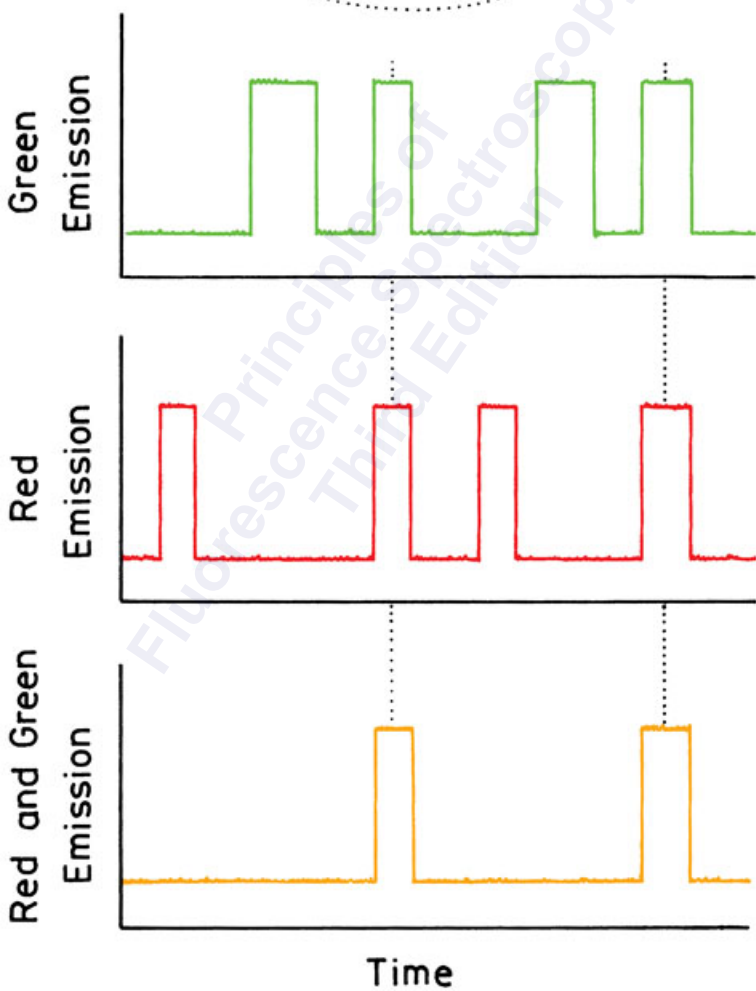
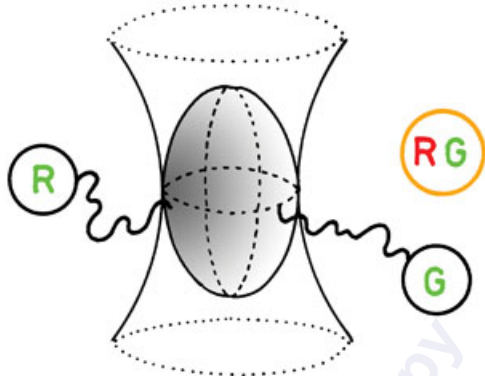
 $\mu\text{m}^2/\text{s}$ 

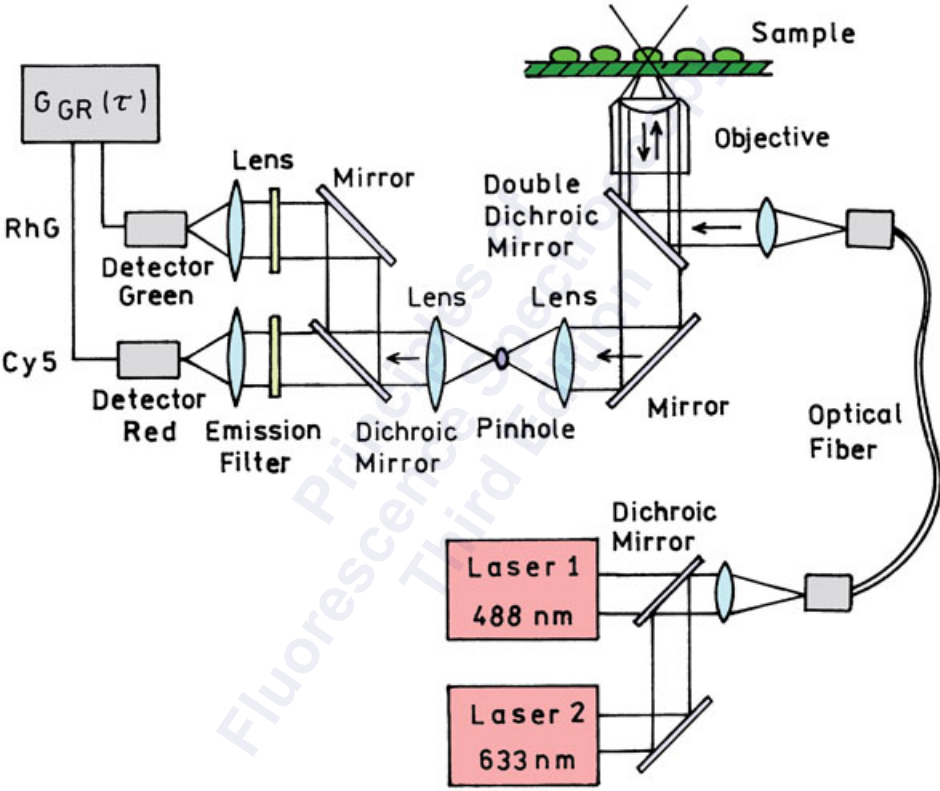
13/0.12
7.9
7.9
8.8
8.2
11.4
14.4
12
12.3
11.2

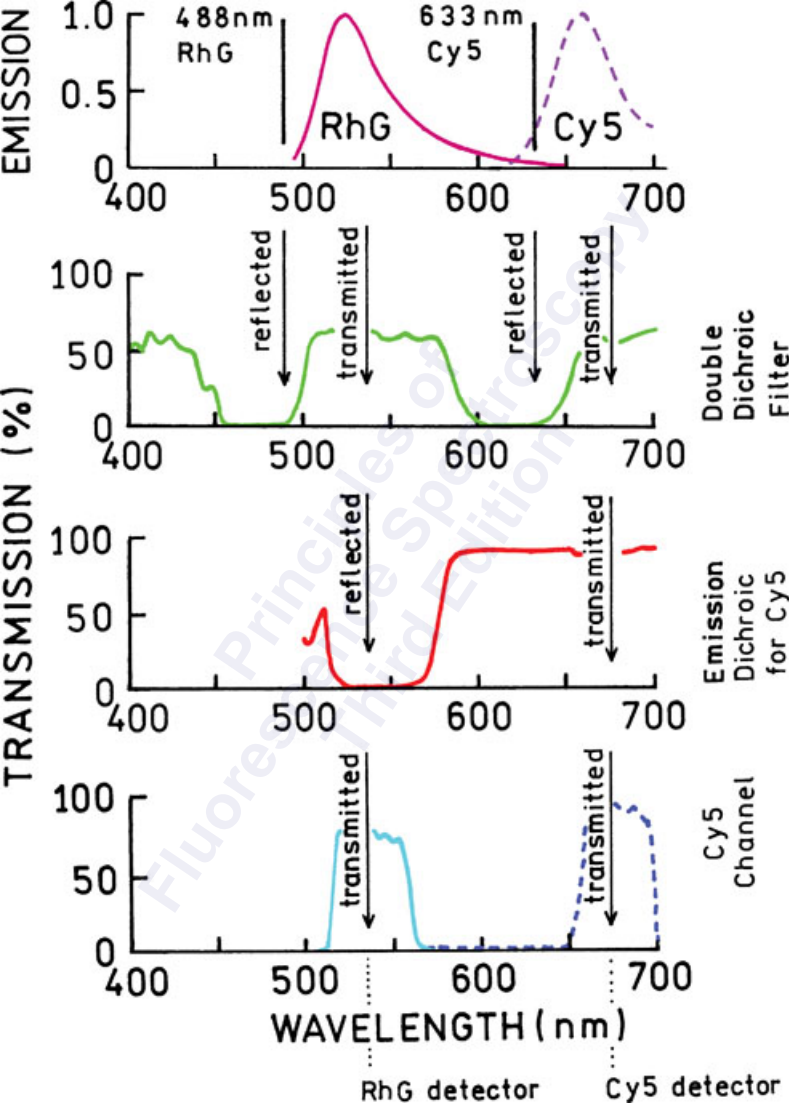
cytoplasm

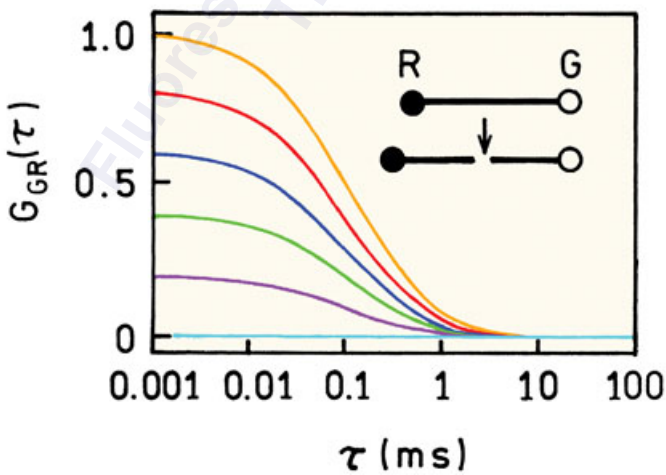
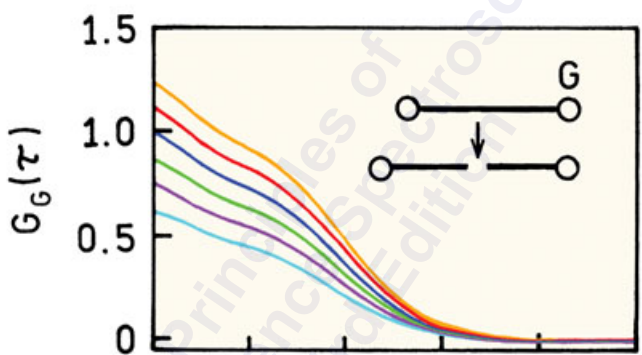
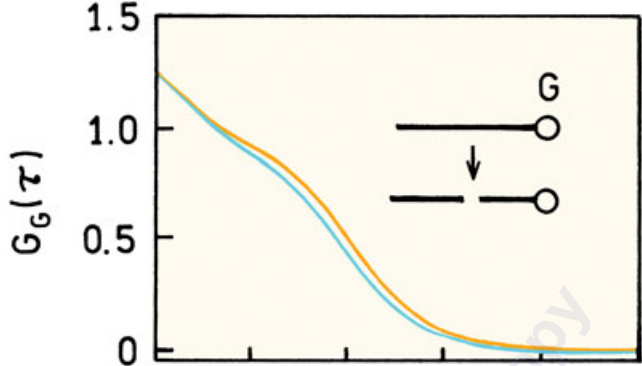
AKM-EGFP





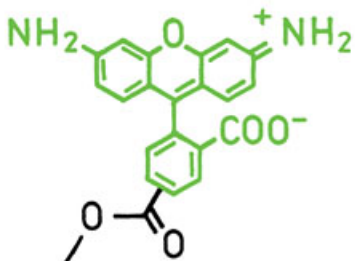




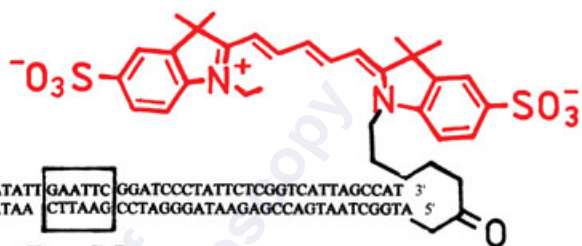




# Rhodamine Green

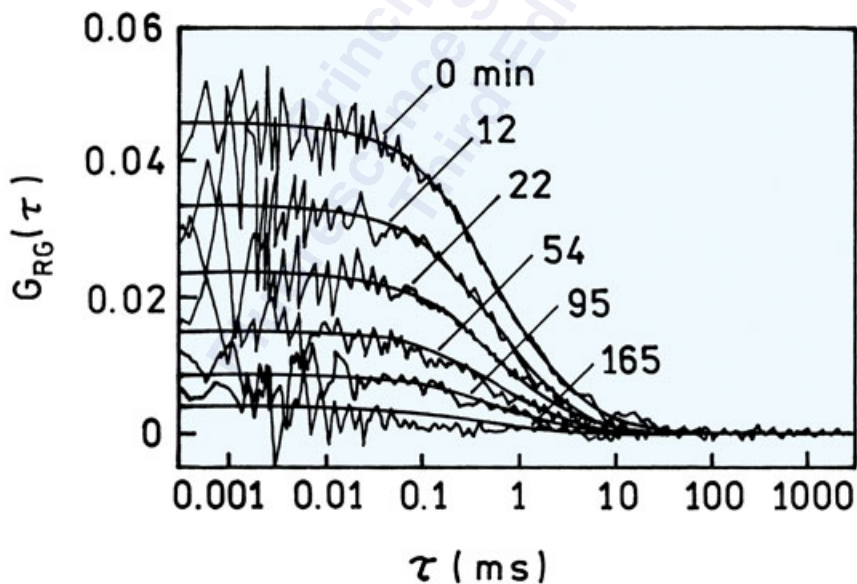


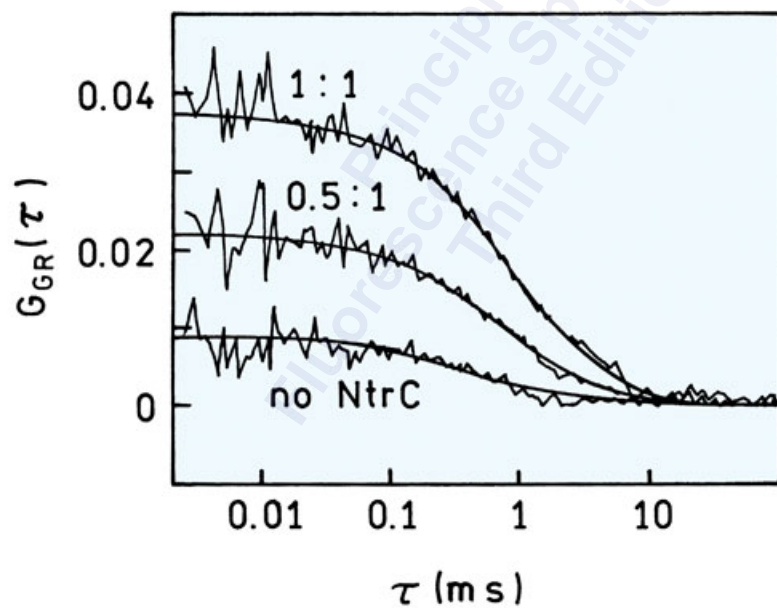
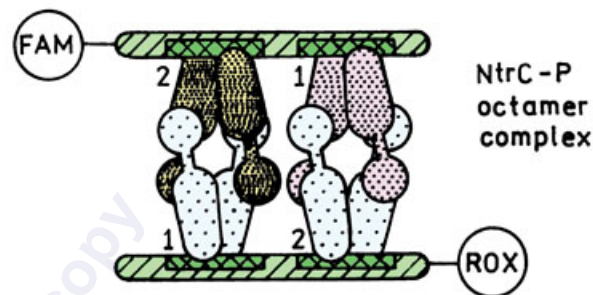
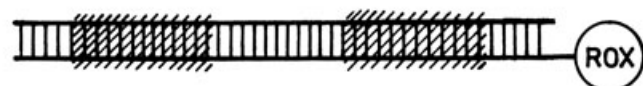
# Cy5



5' GATACGAGCGCAAAGCCCGTAGGTACCAATATTGAATTCGGATCCTATTCTCGGTCATTAGCCAT 3'  
 3' CTATGCTCGCGTTTCGGGCATCCATGGTTATAACTTAAGCCTAGGGATAAGAGCCAGTAATCGGTA 5'

EcoRI







Leukemia cell

RNA  $\longrightarrow$  cDNA

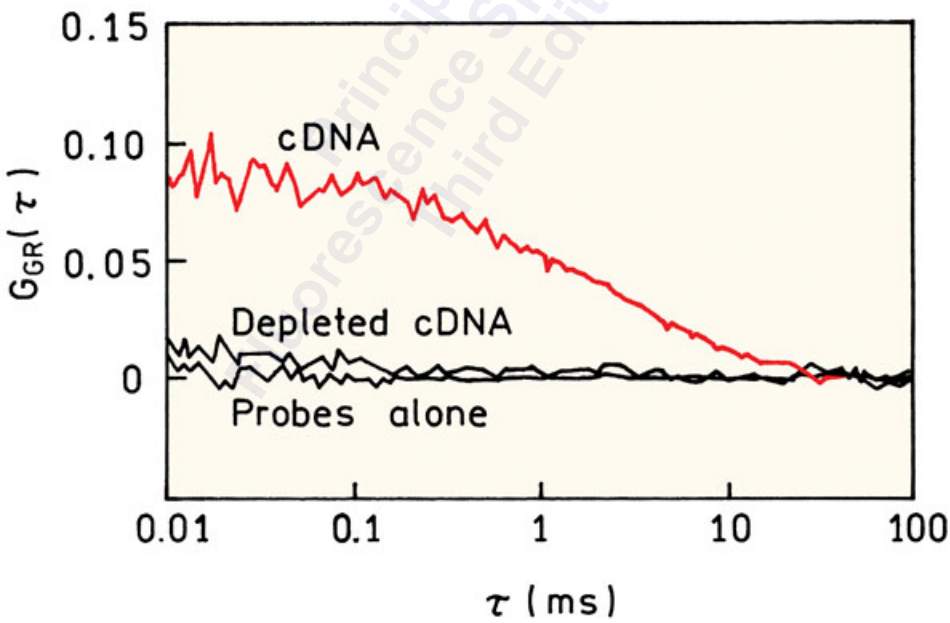
G ○ —

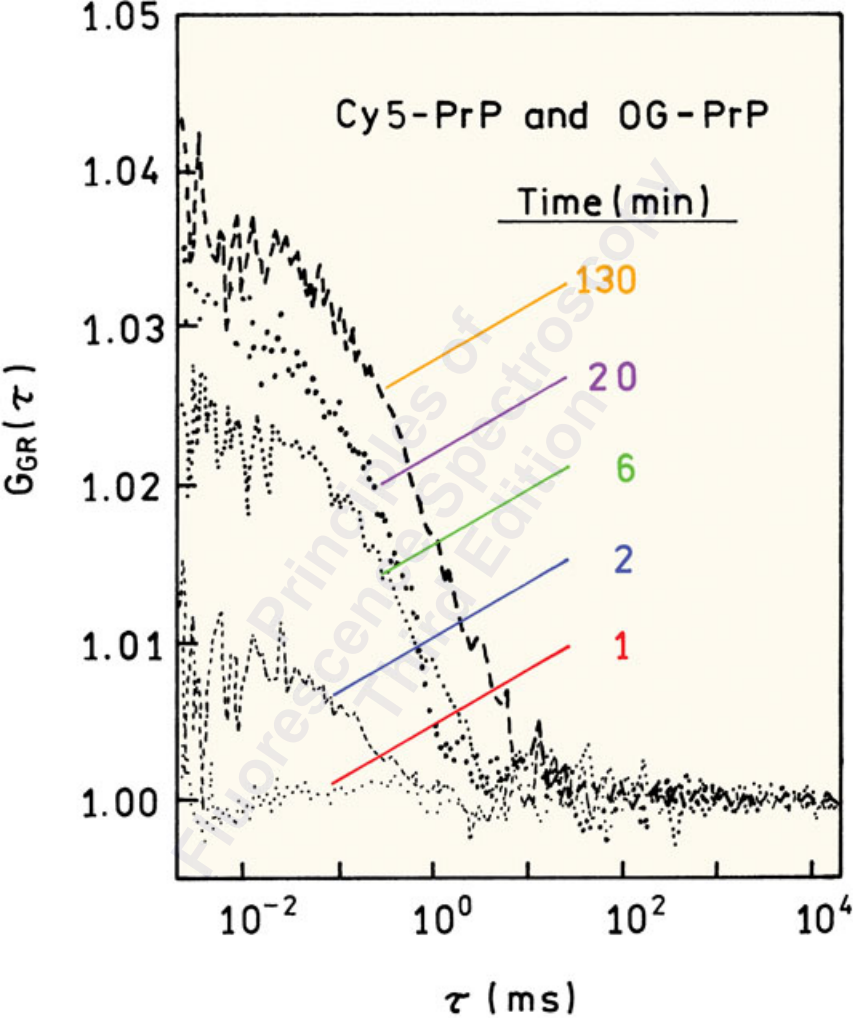
R ● —

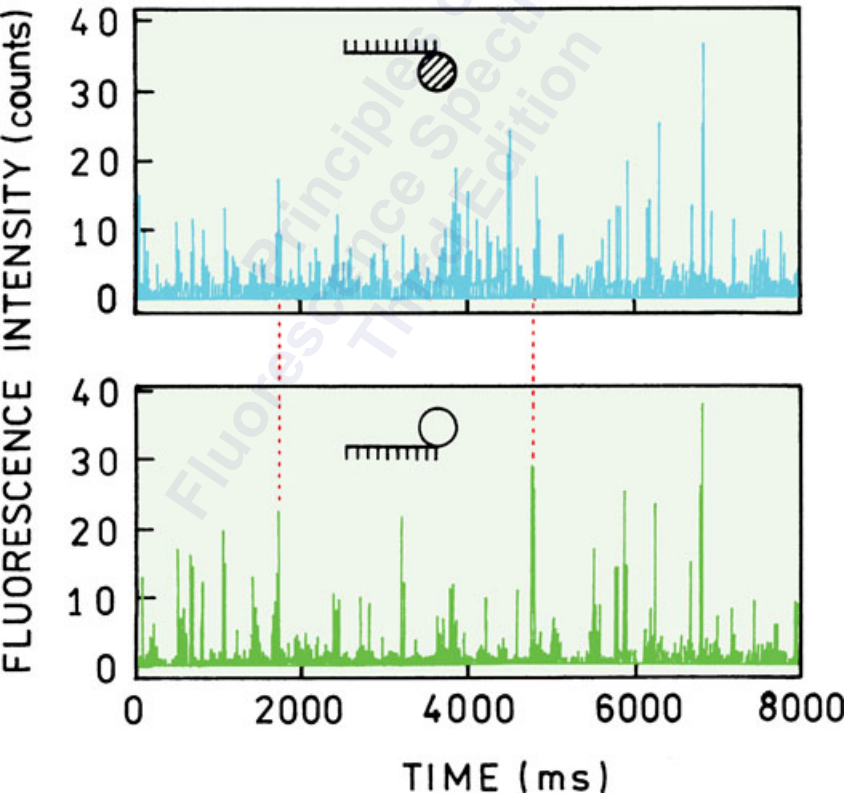
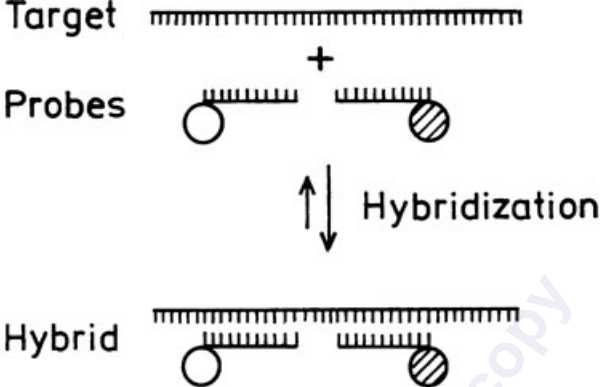
Probes

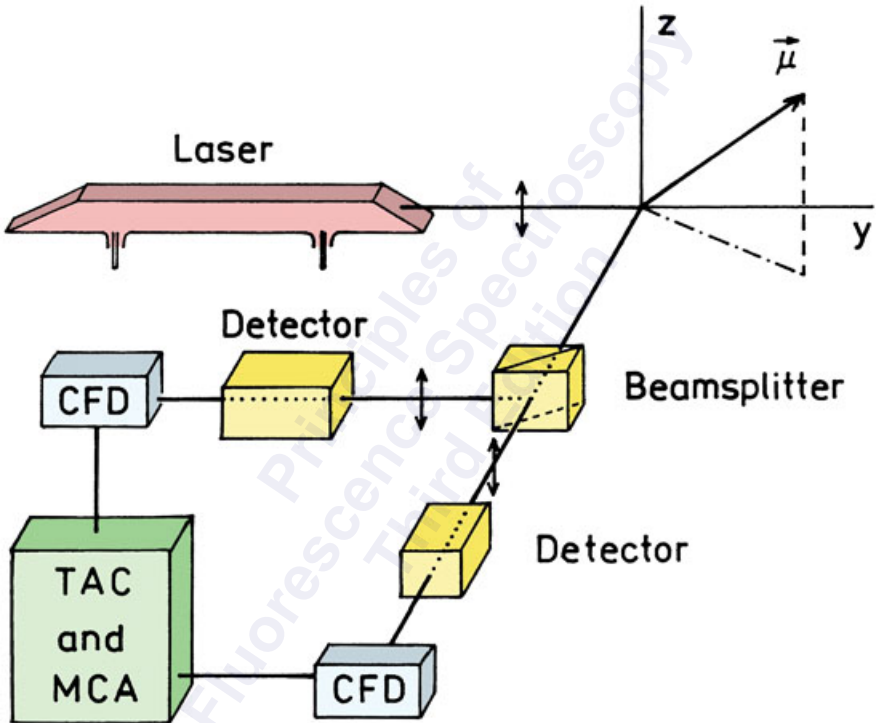
+ cDNA  $\longrightarrow$

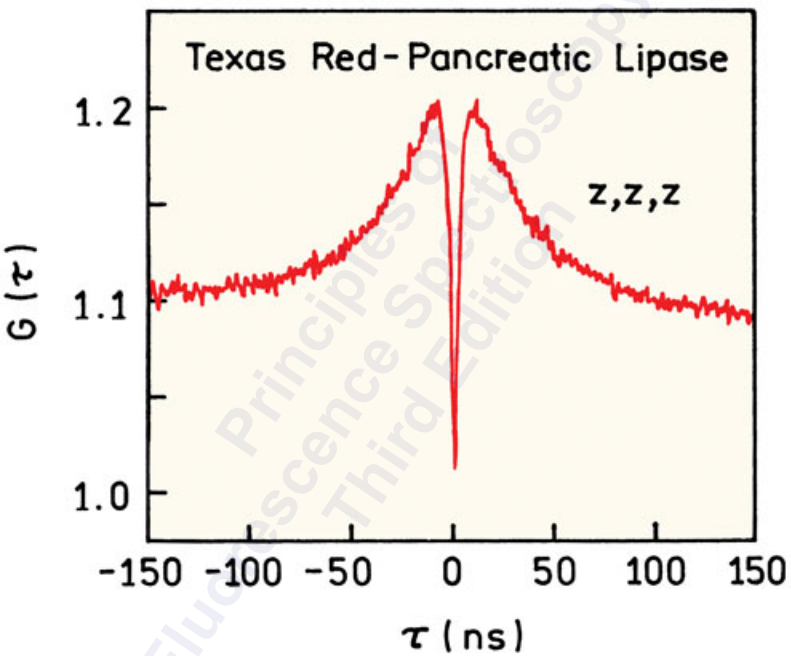
○ — — — — — ●  
Gene

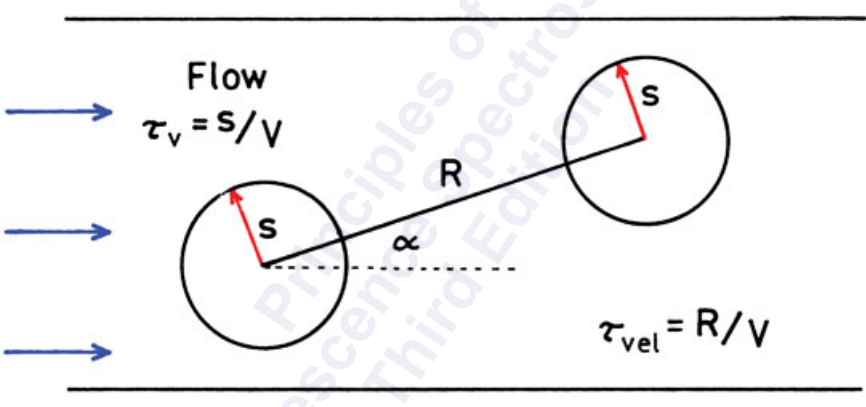




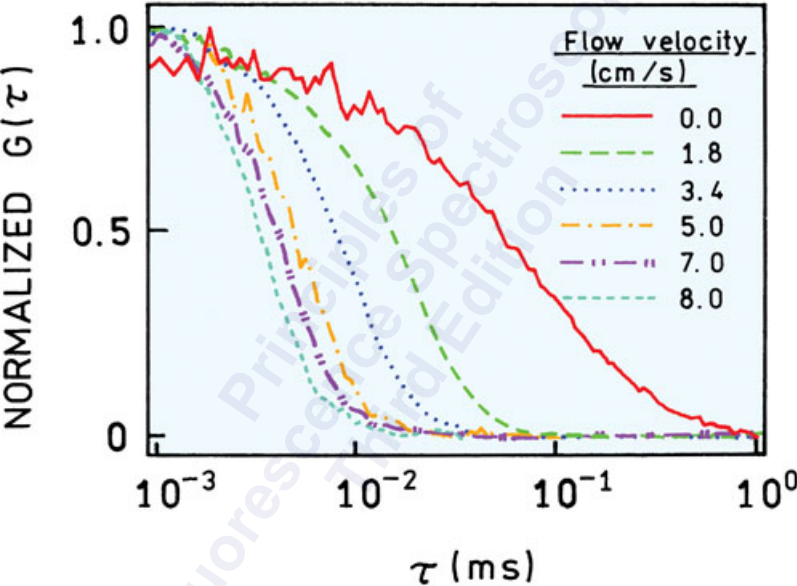




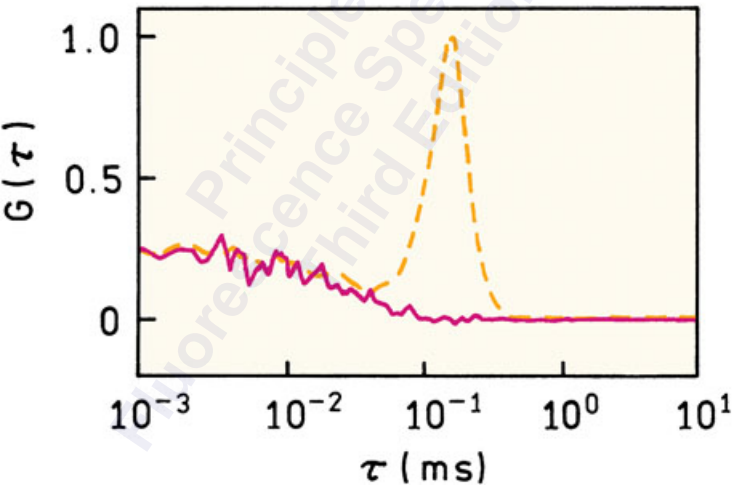
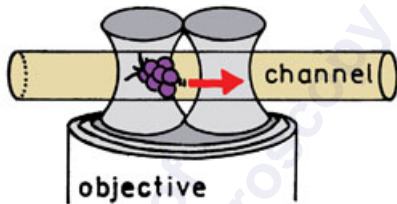


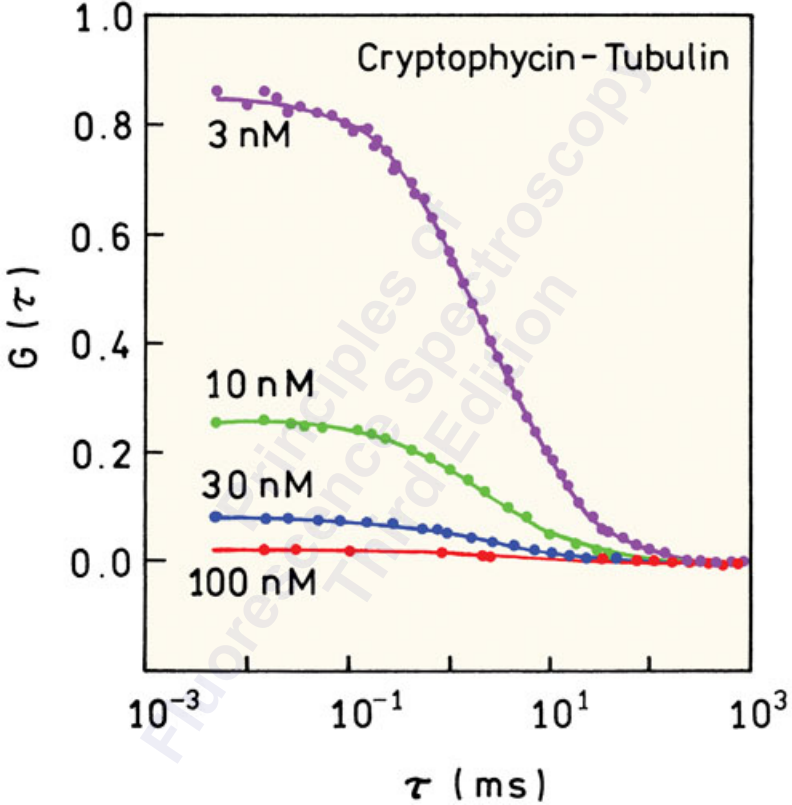


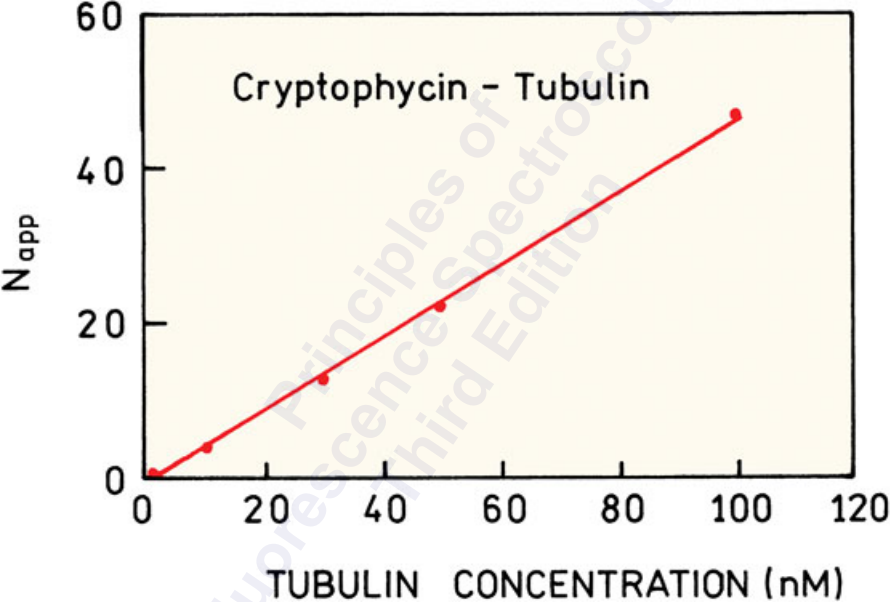


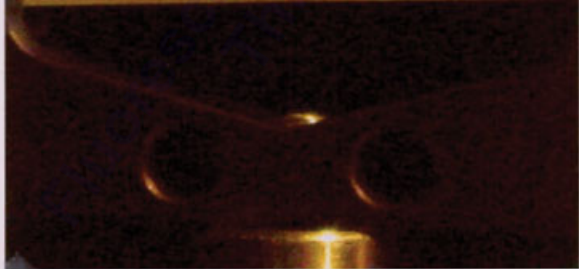
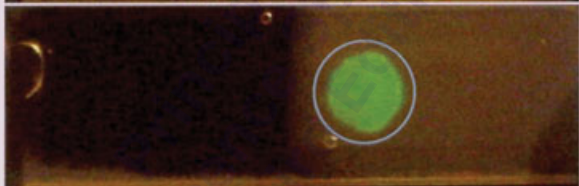
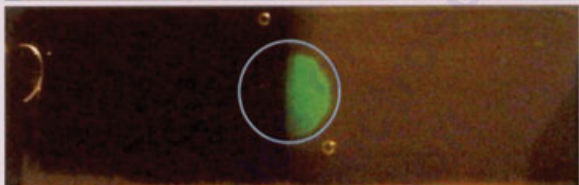


Focussed laser beams

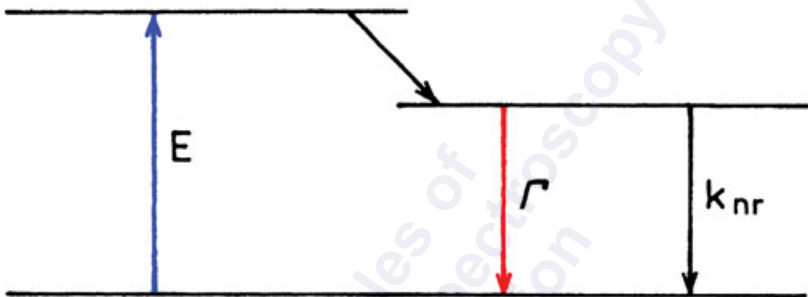




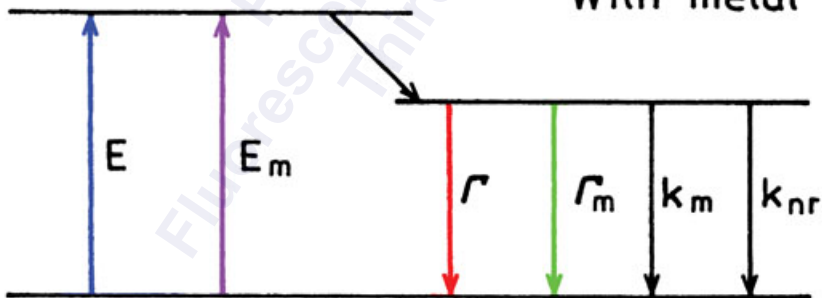


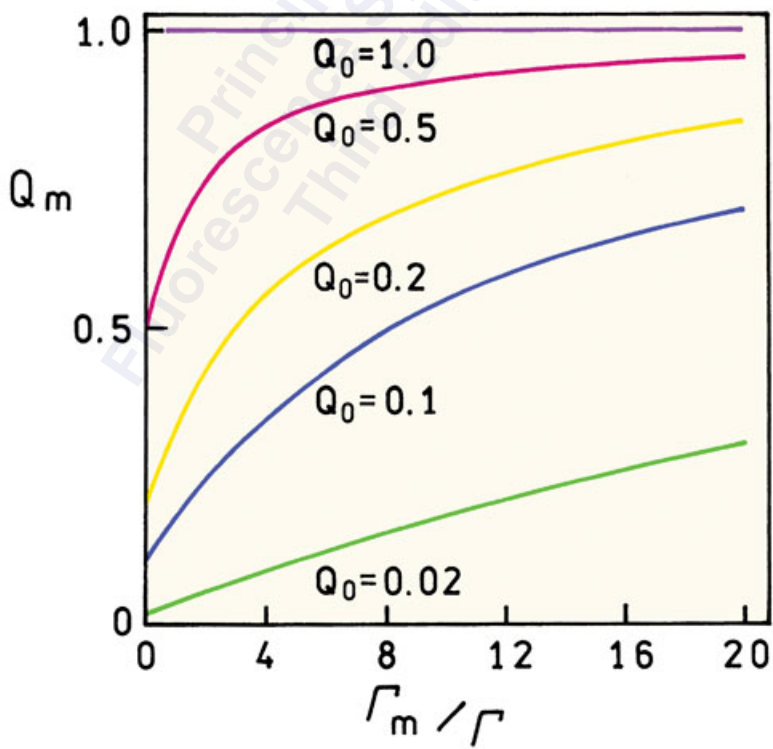
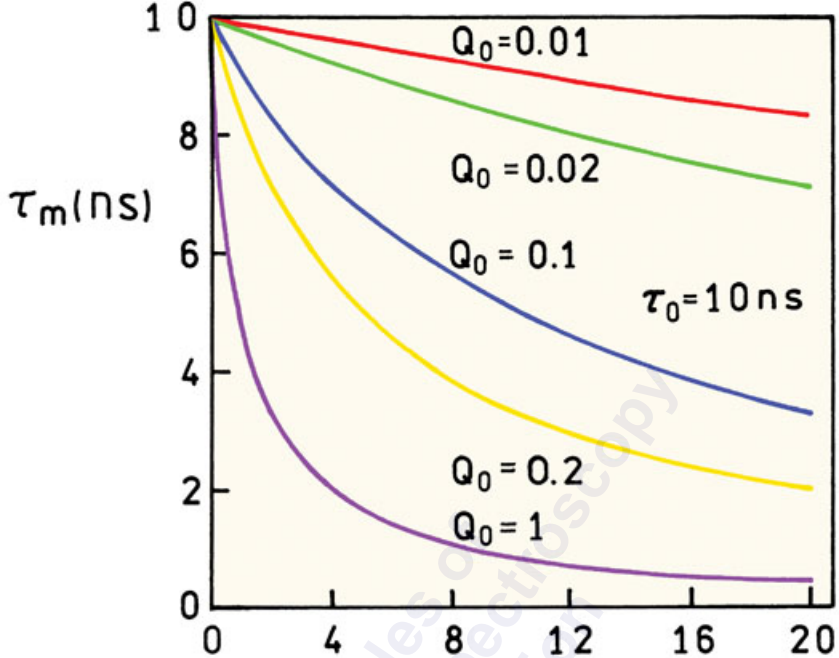


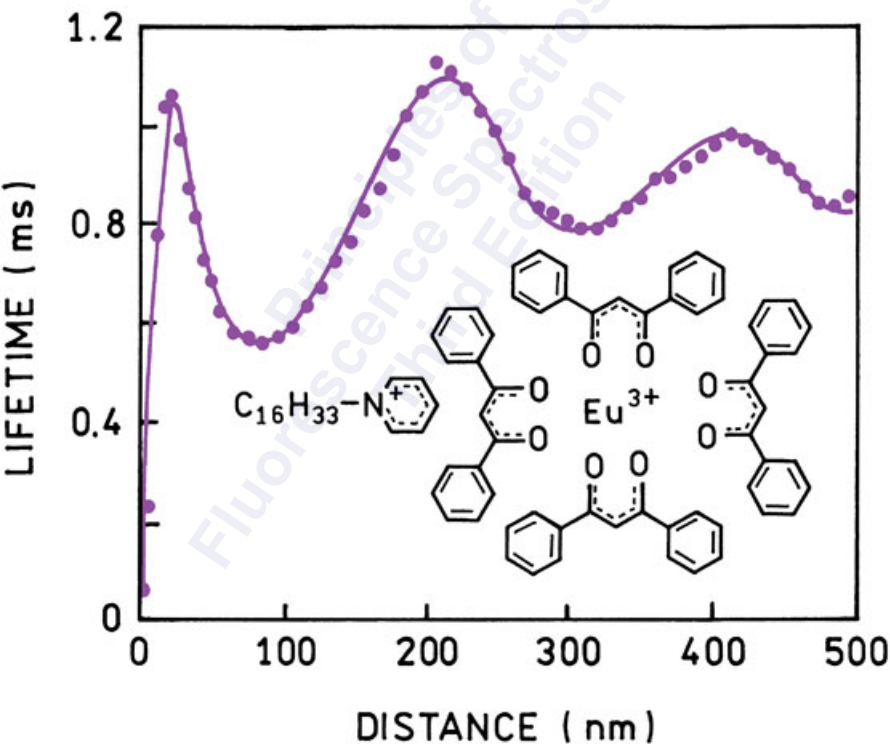
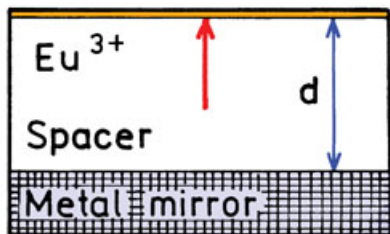
No metal



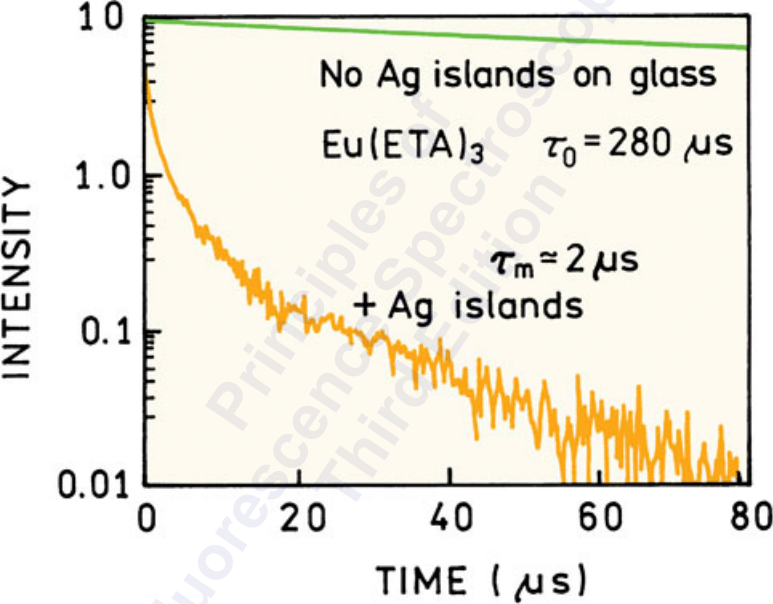
With metal

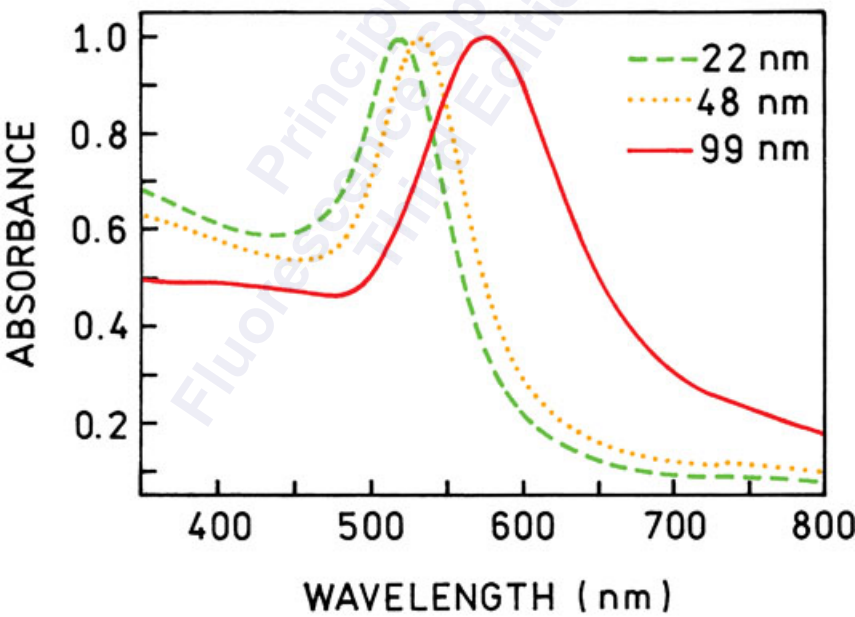
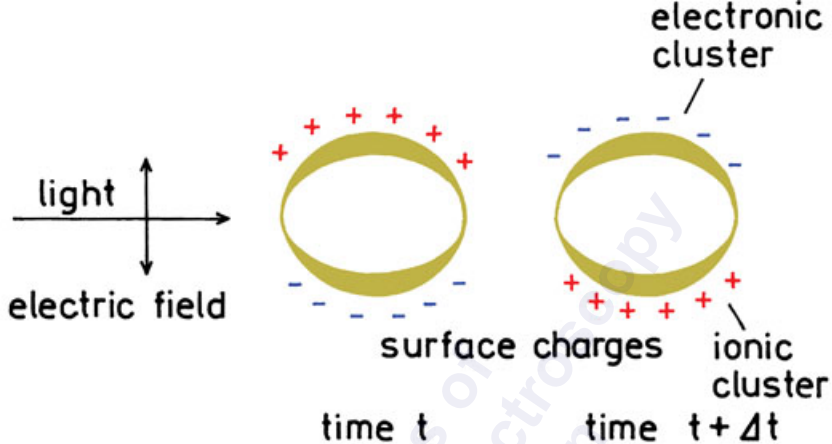




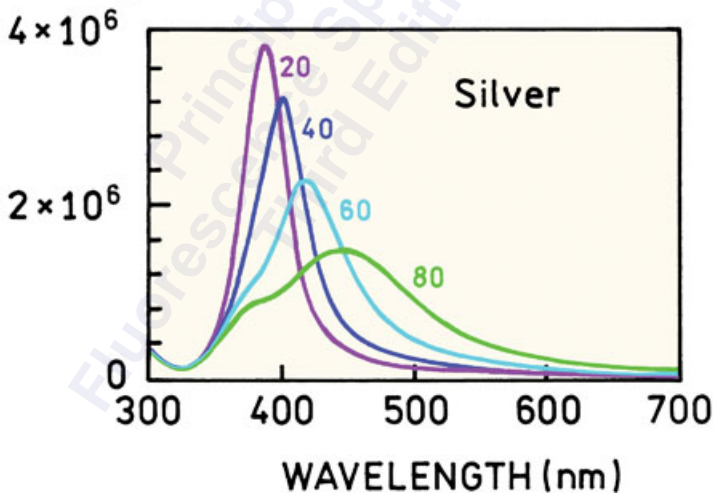
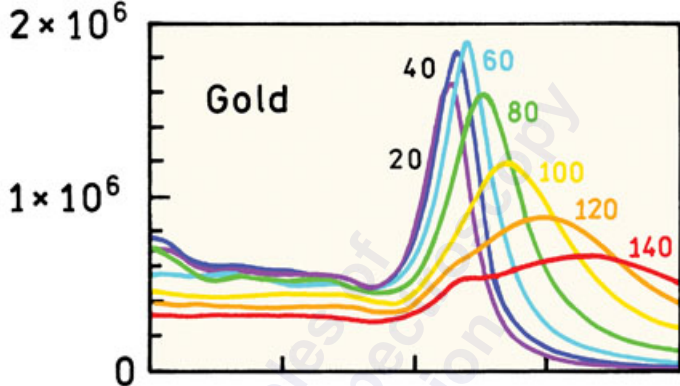


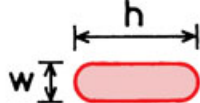






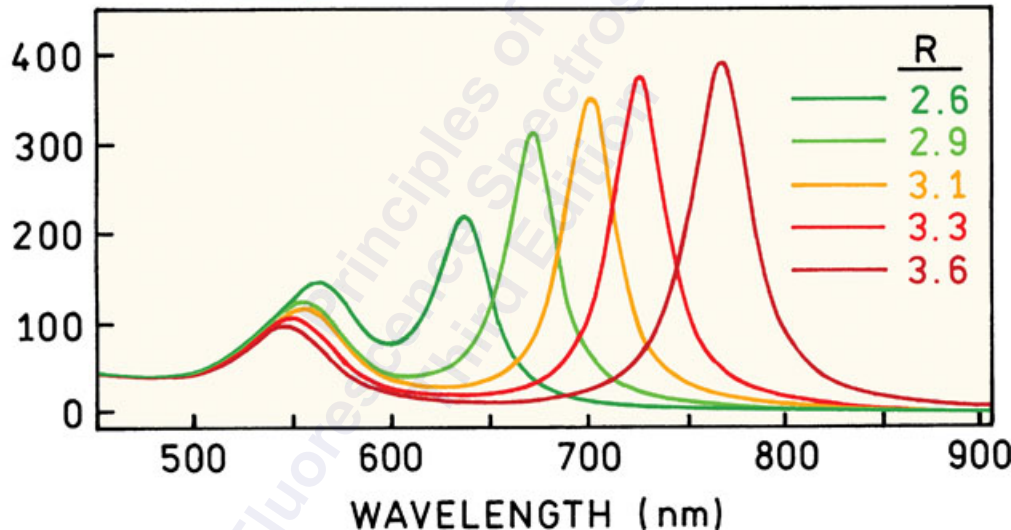
EXTINCTION



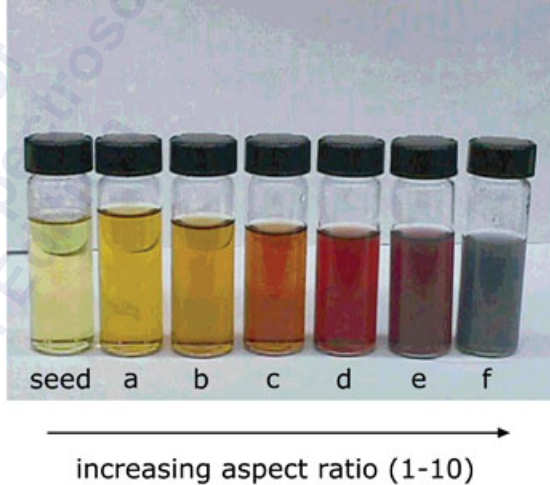
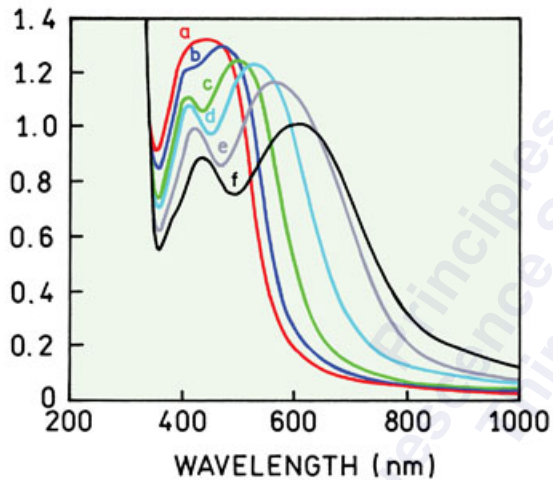


Aspect Ratio  $R = h/w$

ABSORBANCE (a.u.)



ABSORBANCE



40 nm Ag

2 pM



40 nm Au

13 pM



78 nm Au

1.7 pM



118 nm Au

0.5 pM



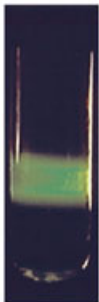
140 nm Au

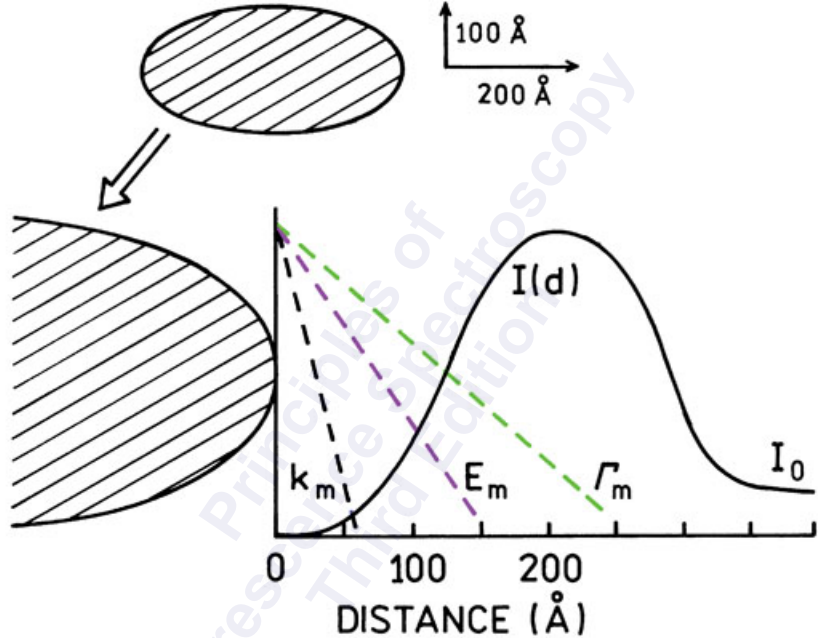
0.3 pM

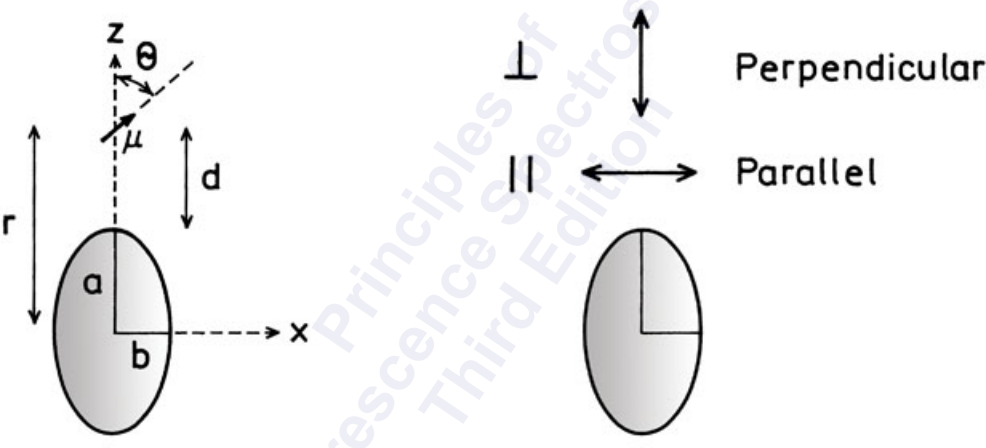


Fluorescein

2  $\mu$ M



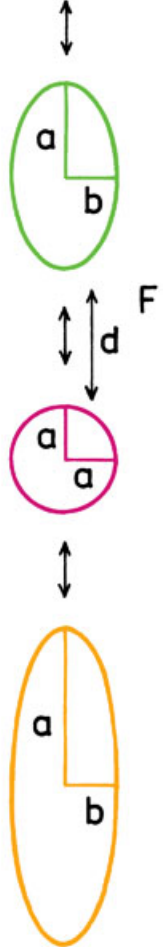
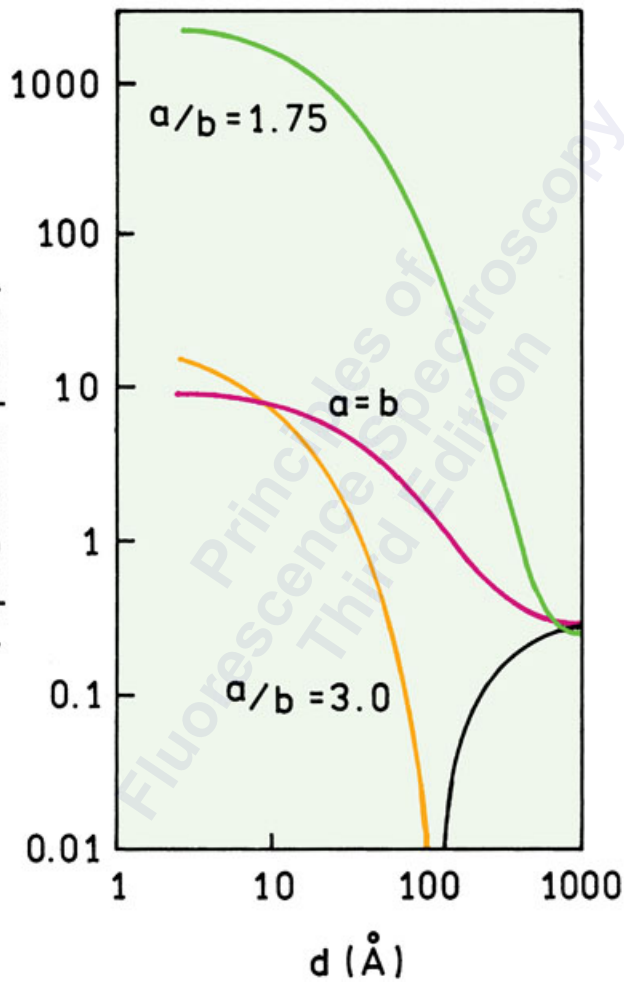


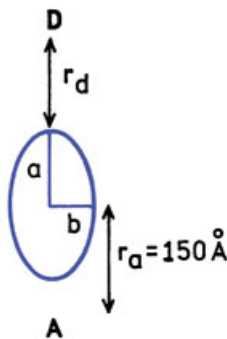
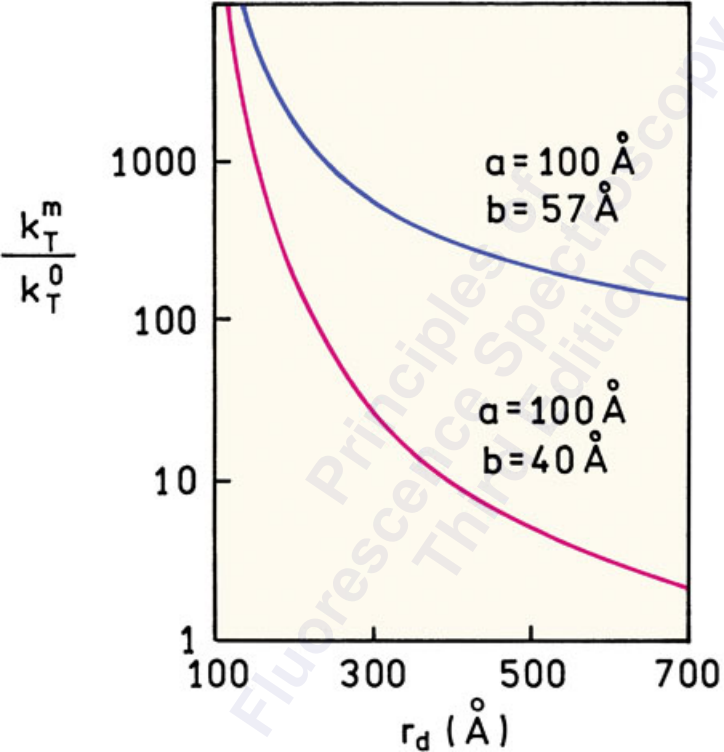


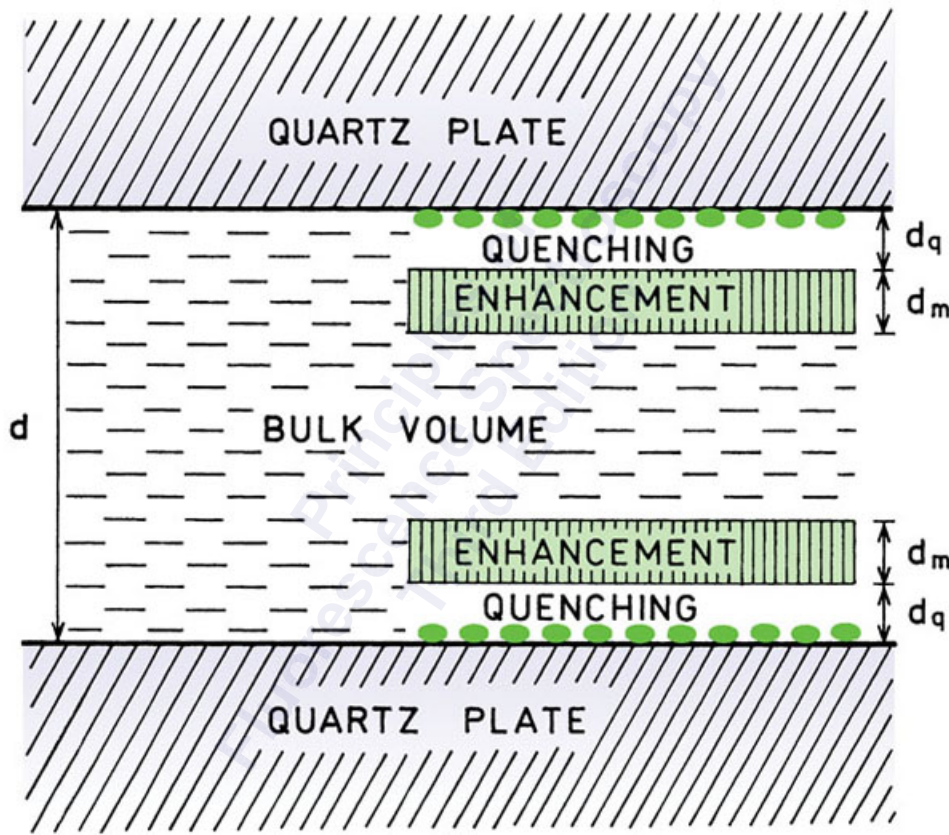


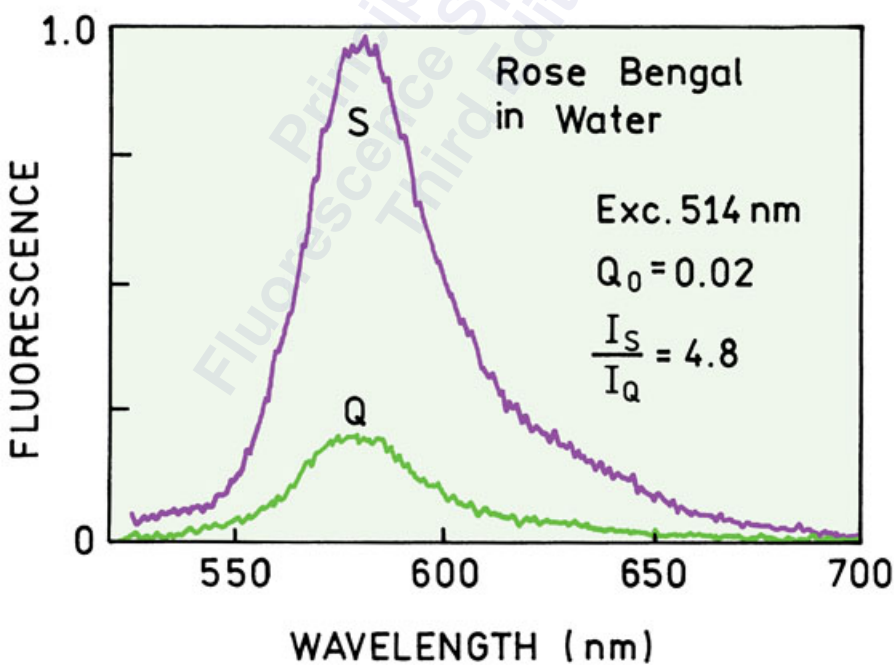
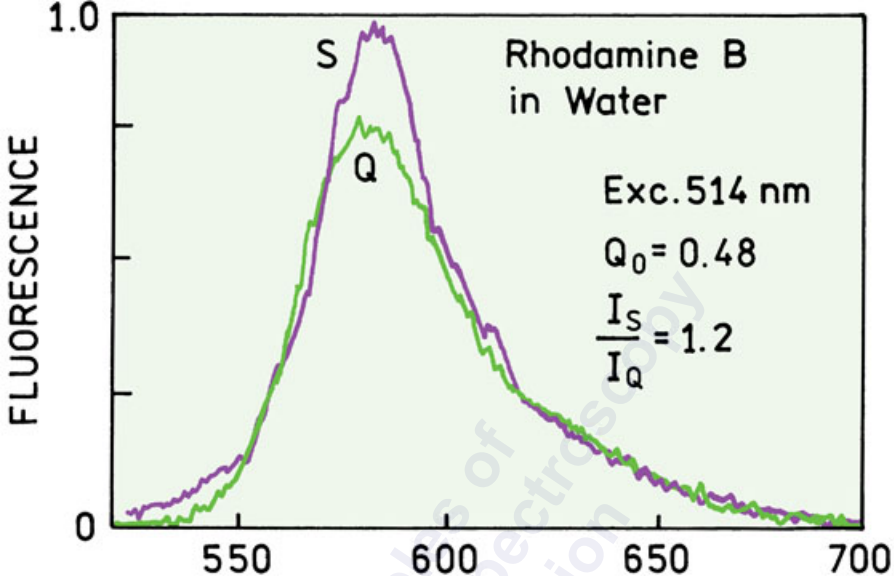
# RATIO OF THE RADIATIVE DECAY RATES

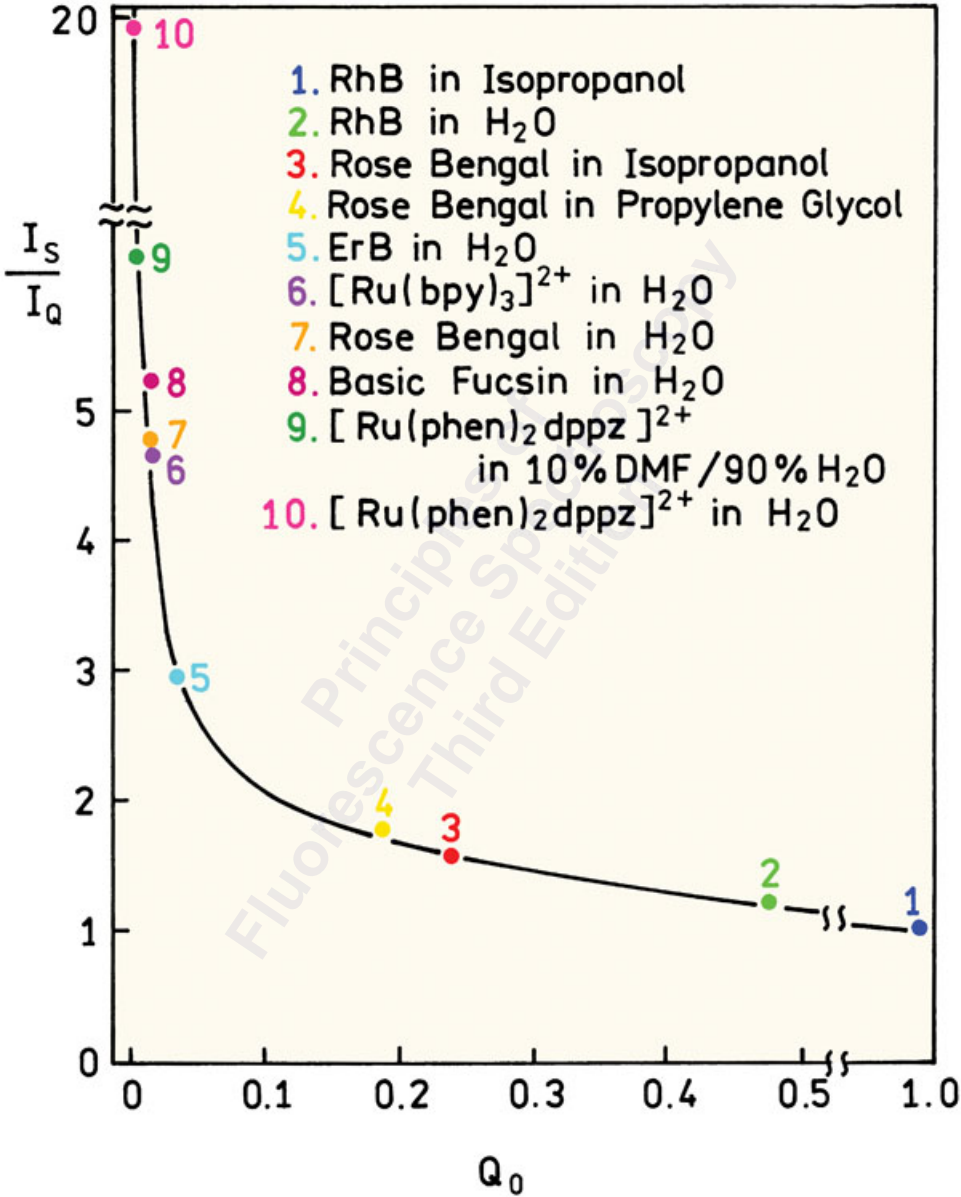
(spherical/plane)

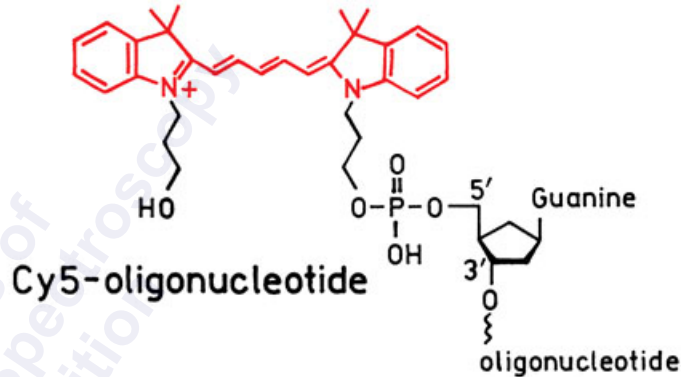
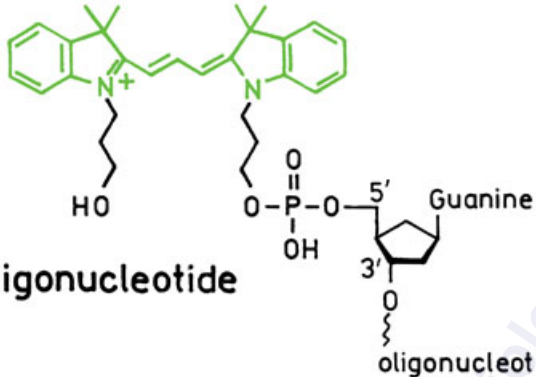






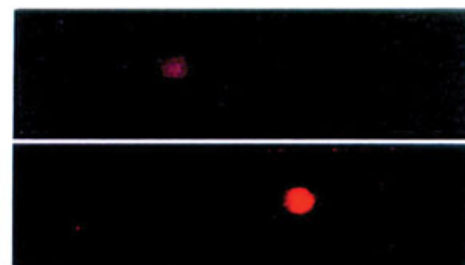
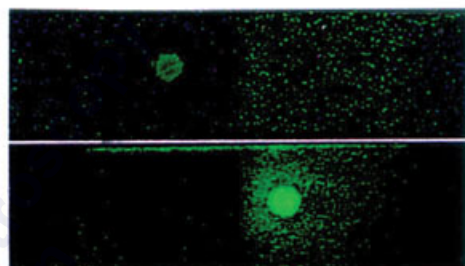
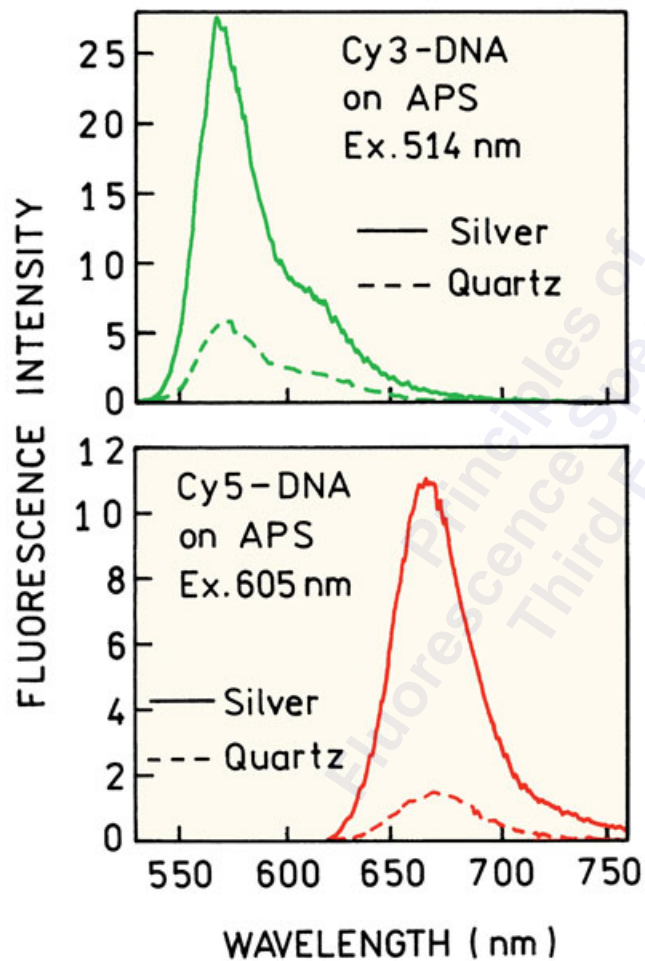




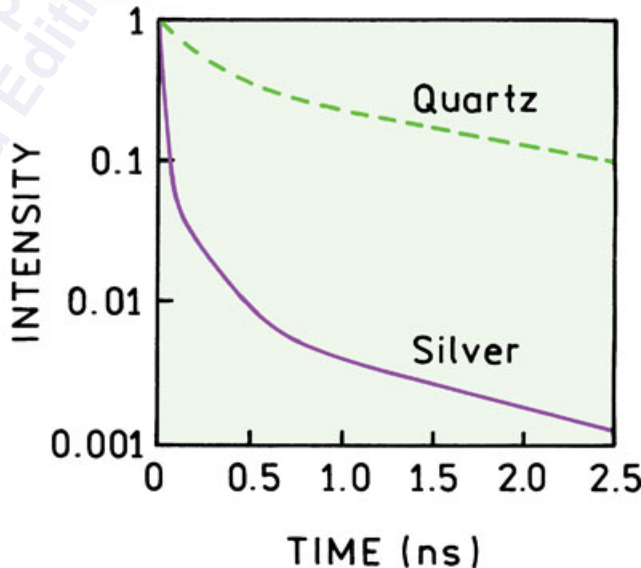
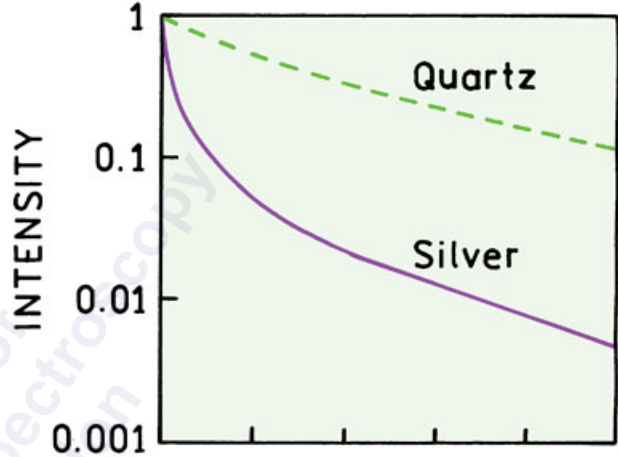
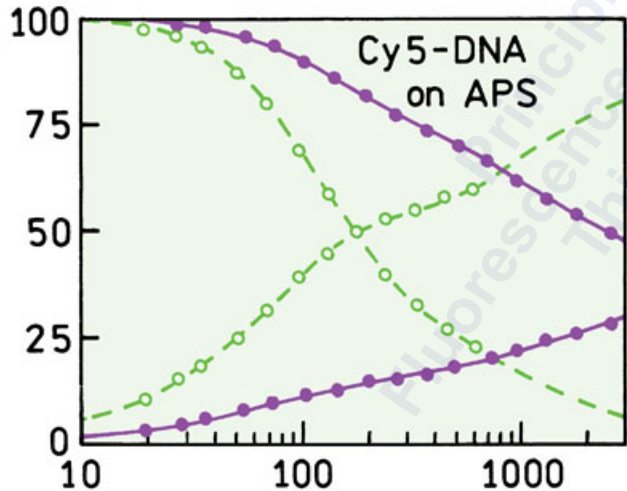
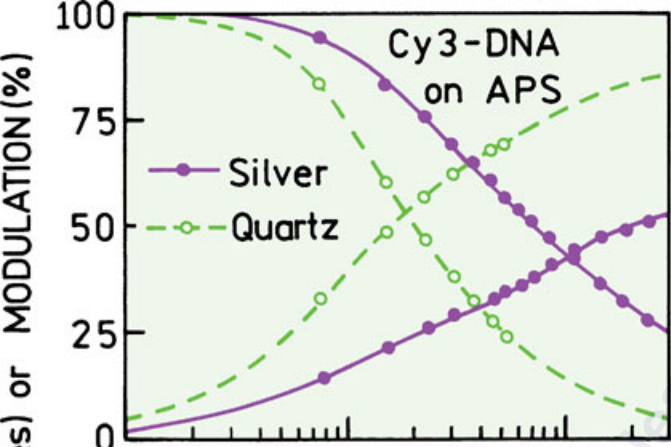


5'- TCC ACA CAC CAC TGG CCA TCT TC-3'  
 3'- AGG TGT GTG GTG ACC GGT AGA AG-5'-Cy3  
 Cy3 - DNA

5'- TCC ACA CAC CAC TGG CCA TCT TC-3'  
 3'- AGG TGT GTG GTG ACC GGT AGA AG-5'-Cy5  
 Cy5 - DNA

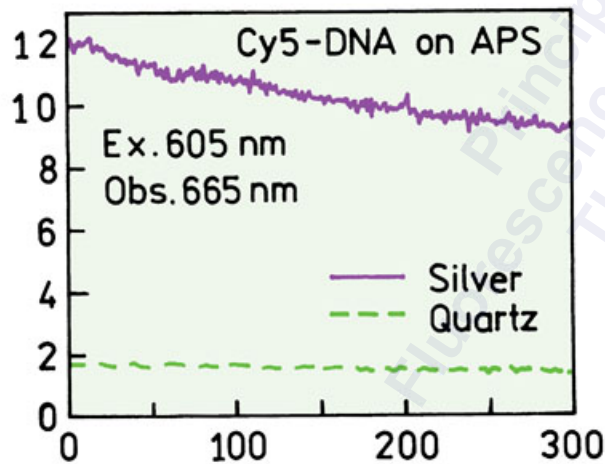
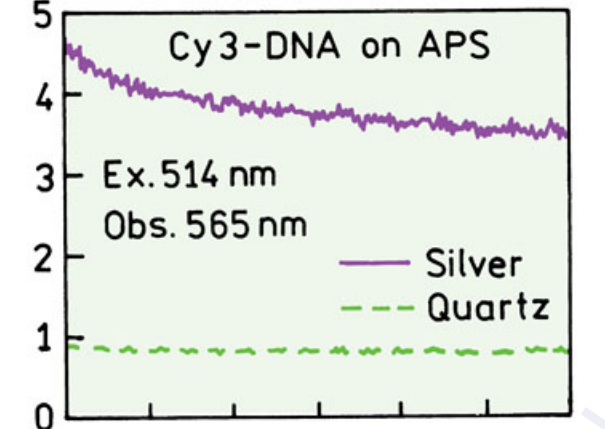




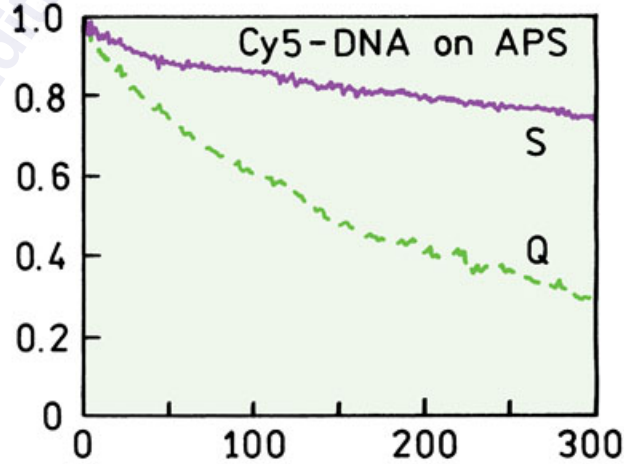
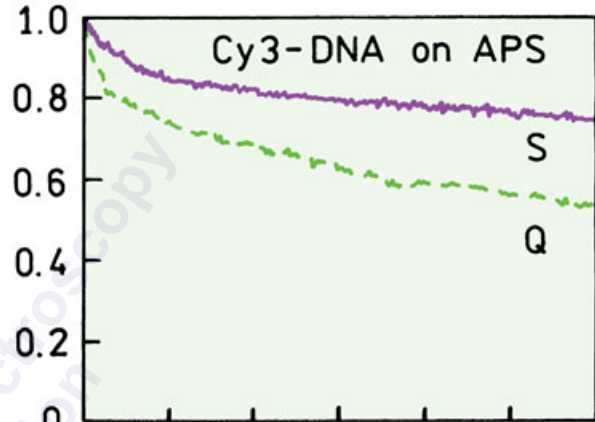




FLUORESCENCE INTENSITY



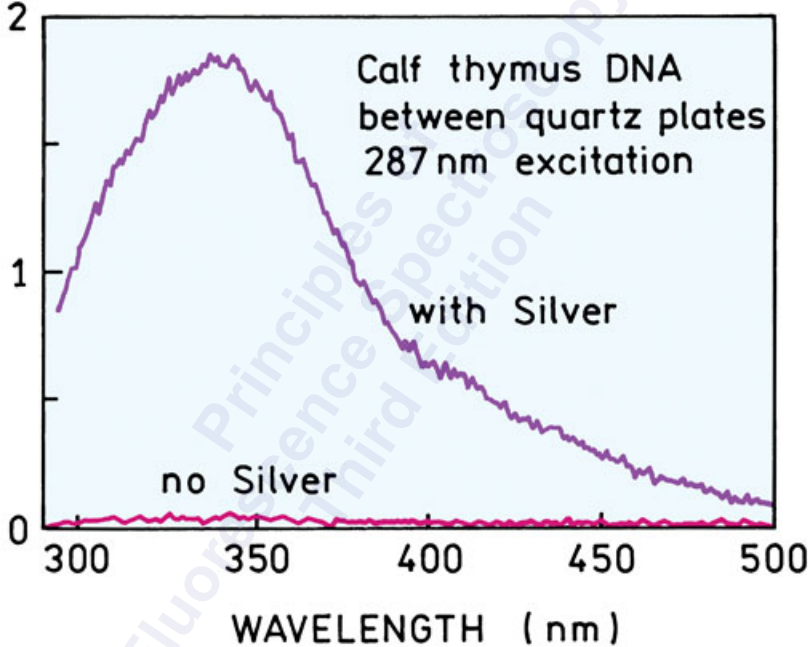
FLUORESCENCE INTENSITY



TIME (s)

TIME (s)

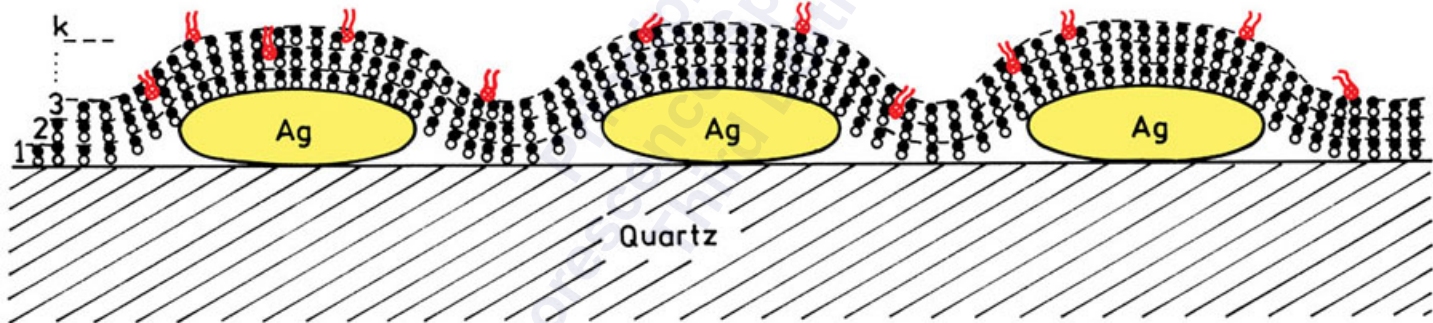
FLUORESCENCE



○ BSA - biotin

● avidin

🔥 DNA(Cy3)-biotin or DNA(Cy5)-biotin

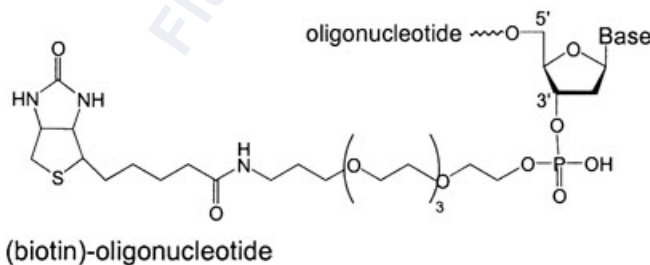
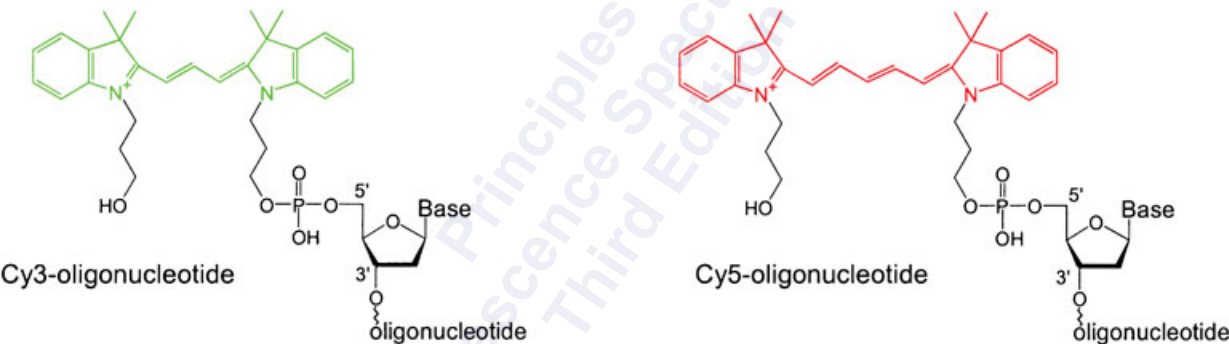


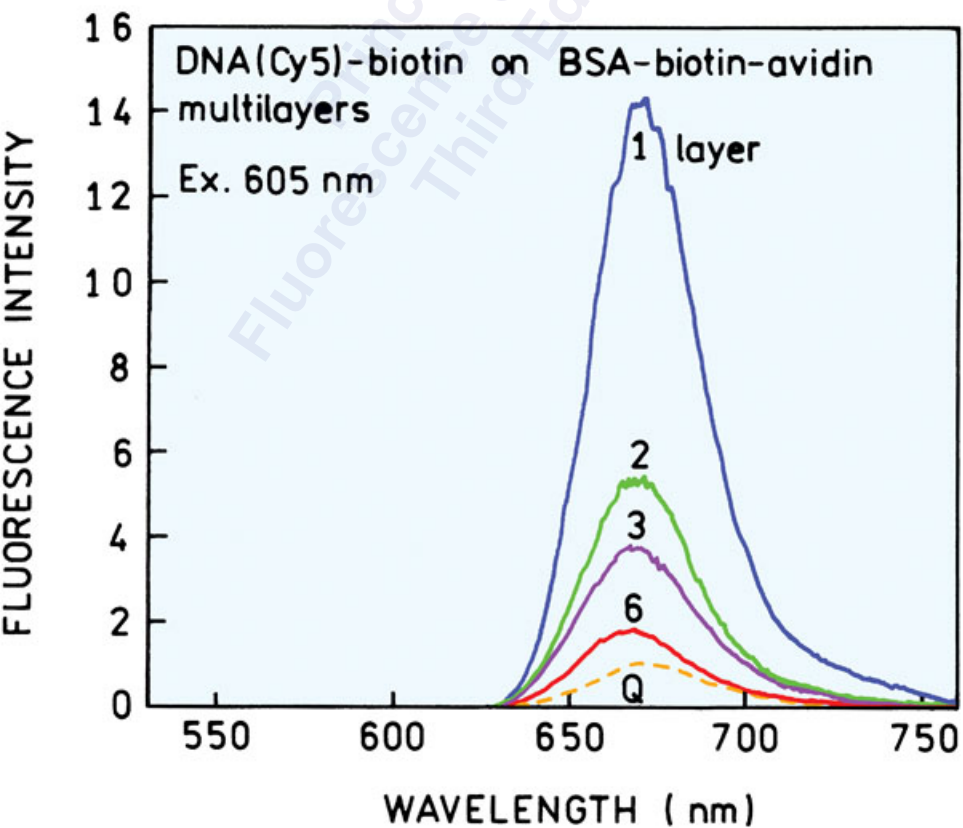
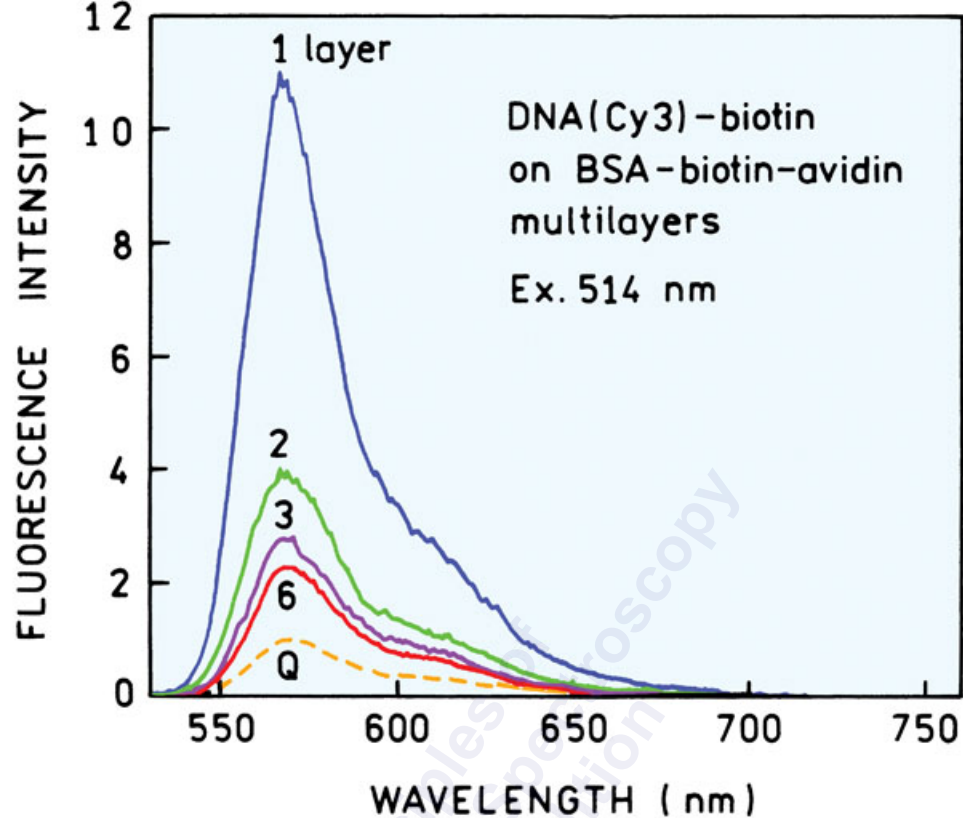
5'-GAA GAT GGC CAG TGG TGT GTG GA-3'-biotin  
 3'-CTT CTA CCG GTC ACC ACA CAC CT-5'-**Cy3**

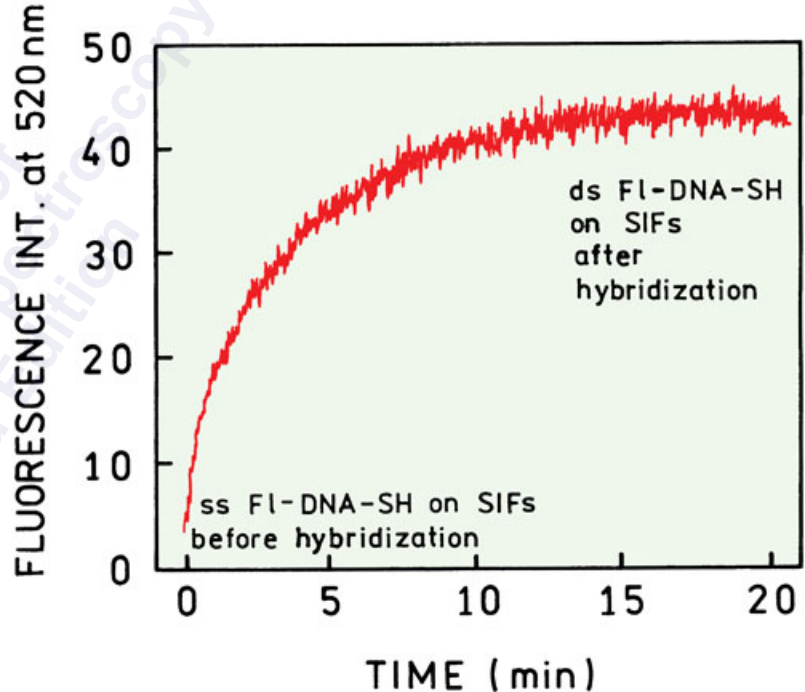
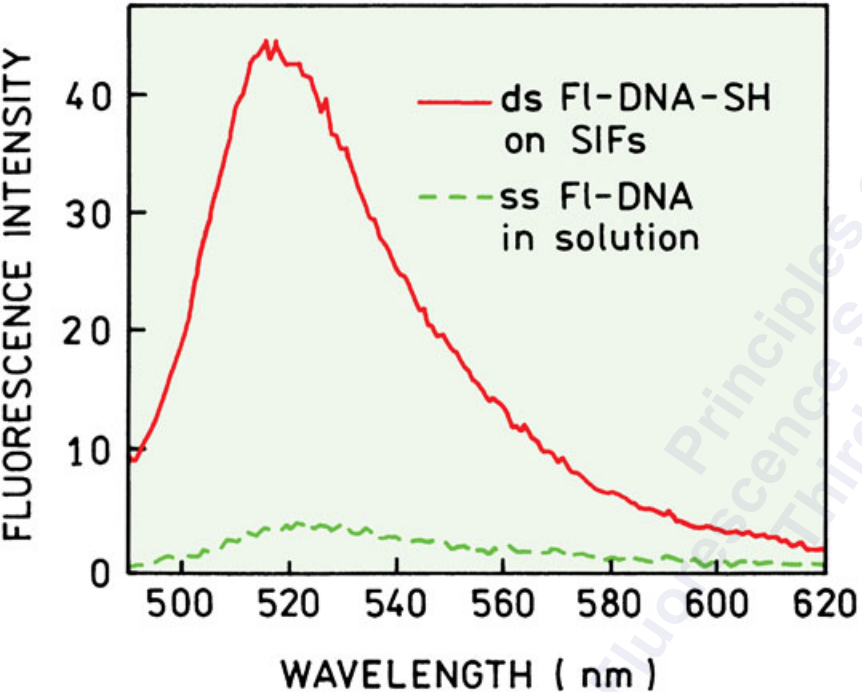
***DNA(Cy3)-biotin***

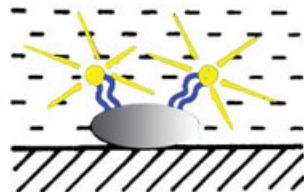
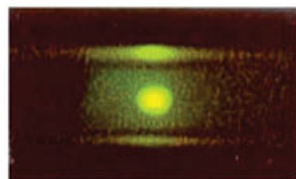
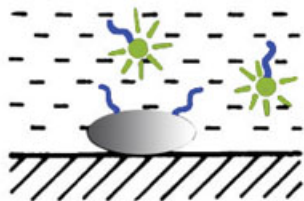
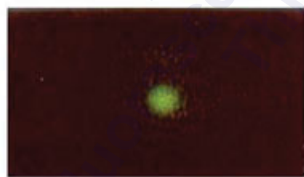
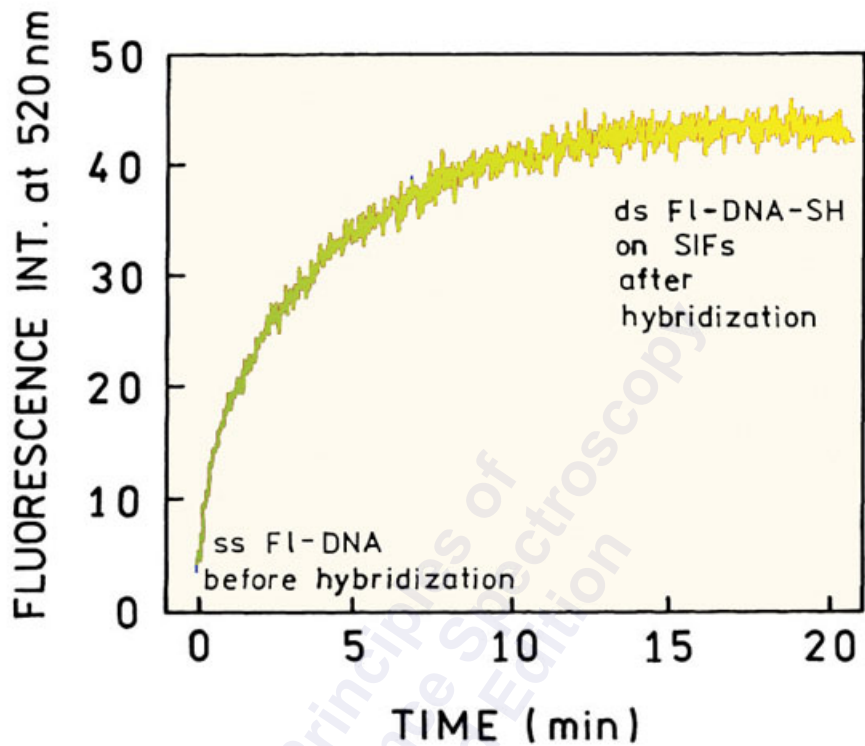
5'-TCC ACA CAC CAC TGG CCA TCT TC-3'-biotin  
 3'-AGG TGT GTG GTG ACC GGT AGA AG-5'-**Cy5**

***DNA(Cy5)-biotin***











FLUORESCENCE INTENSITY

Exc. 488 nm

$L = 0.7$

8.1

12.7

25.6

FITC - IgG  
in Cuvette

ANISOTROPY

LABELING

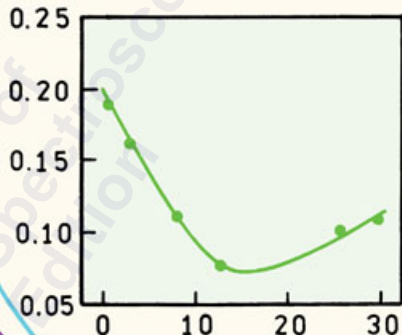
500

540

580

620

WAVELENGTH (nm)

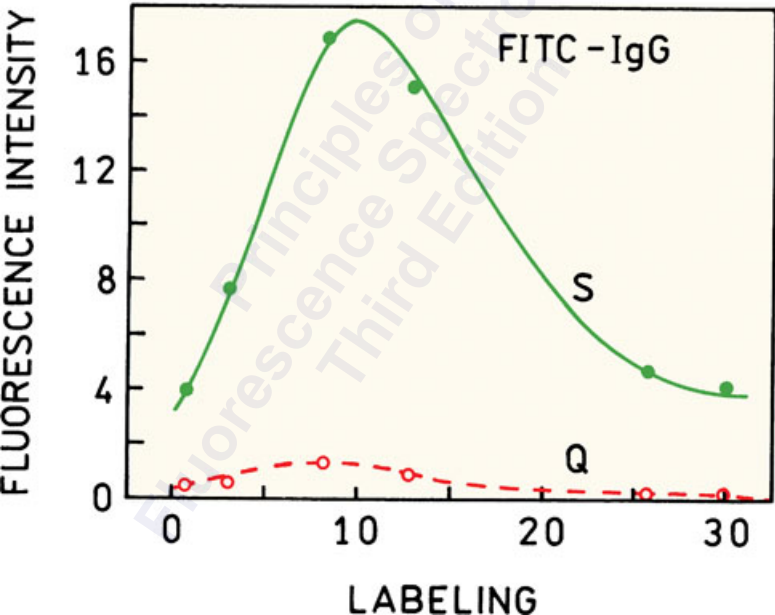




Q

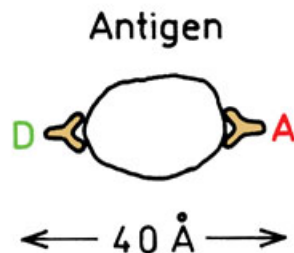
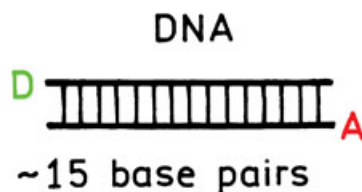


S



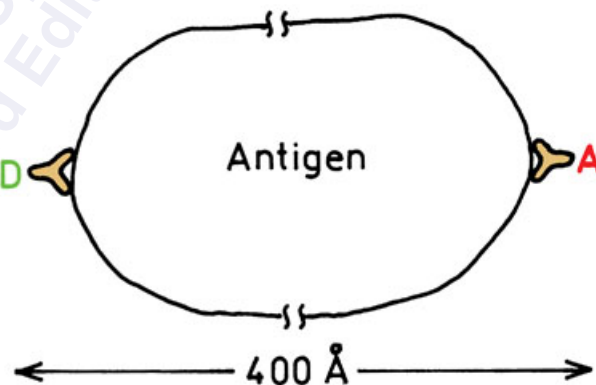
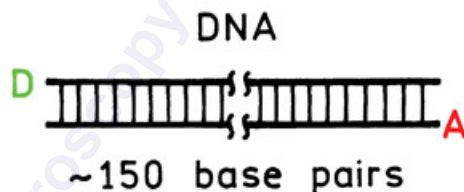
E = 50 %

No Silver



In absence of silver islands  
energy transfer occurs  
within tens of Angstroms

With Silver



In presence of silver islands  
energy transfer occurs  
within hundreds of Angstroms

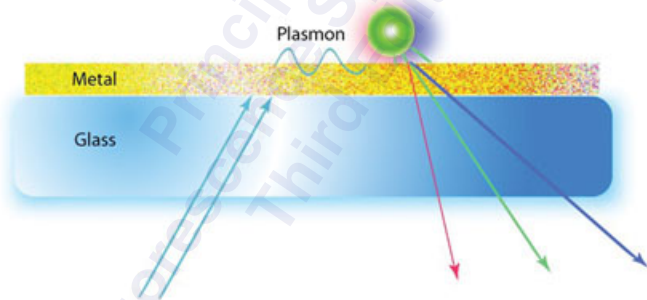


## Fluorescence Today

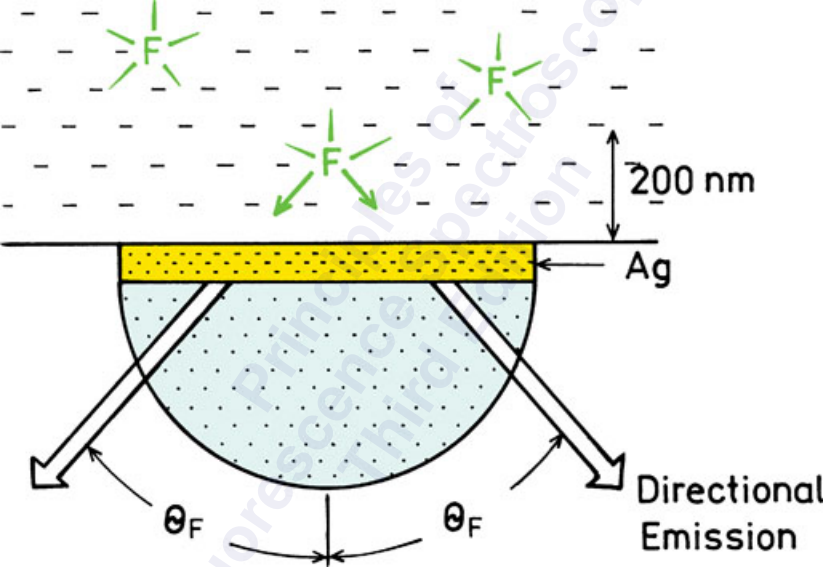
Free-space emission

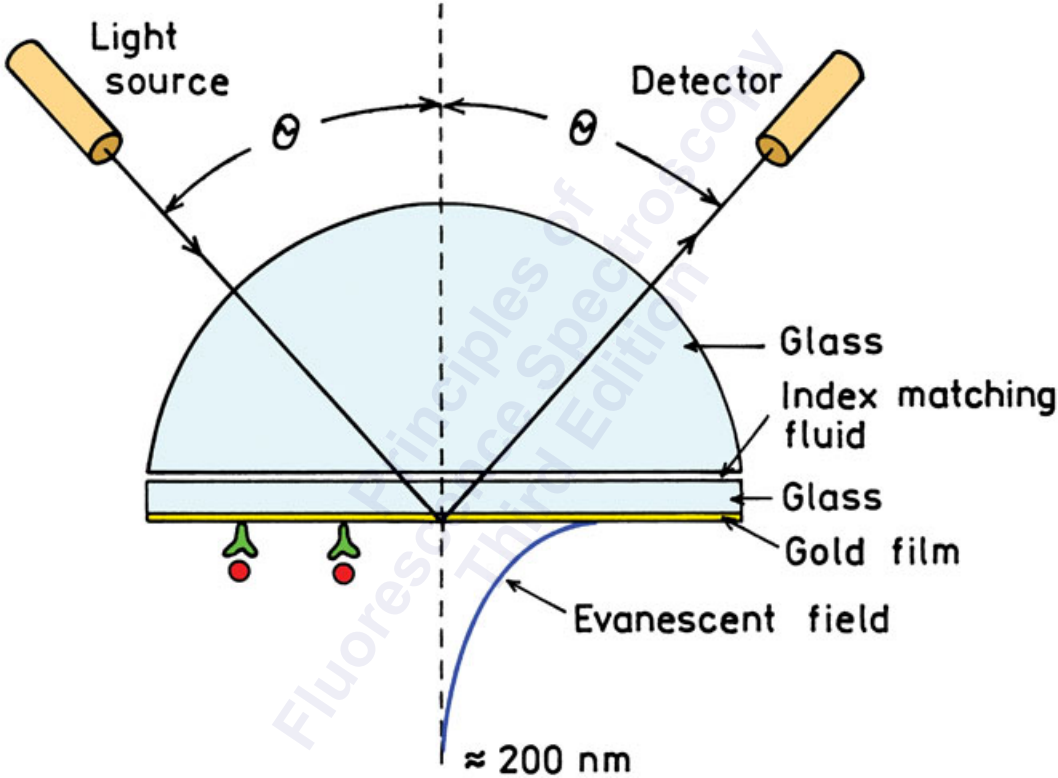


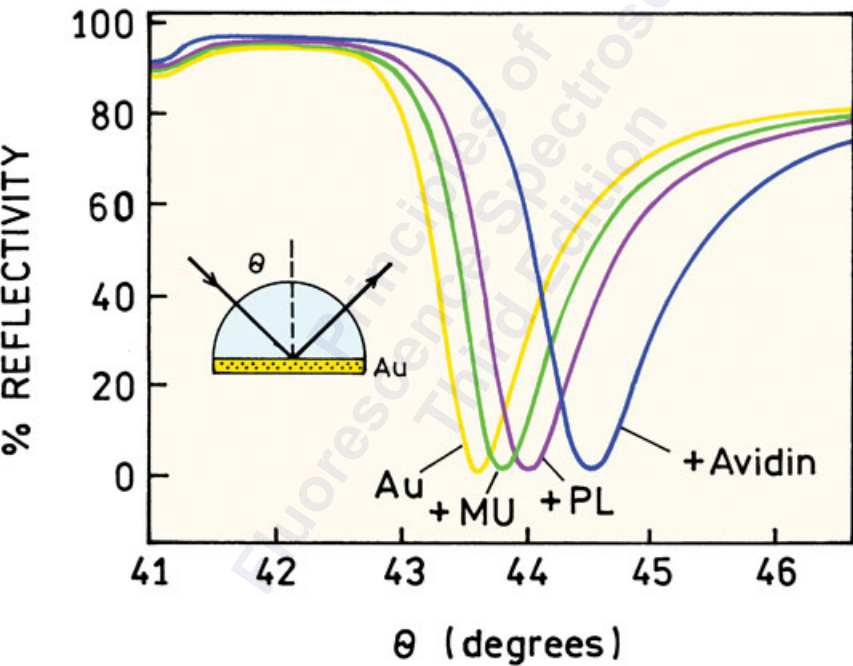
## Radiative Decay Engineering

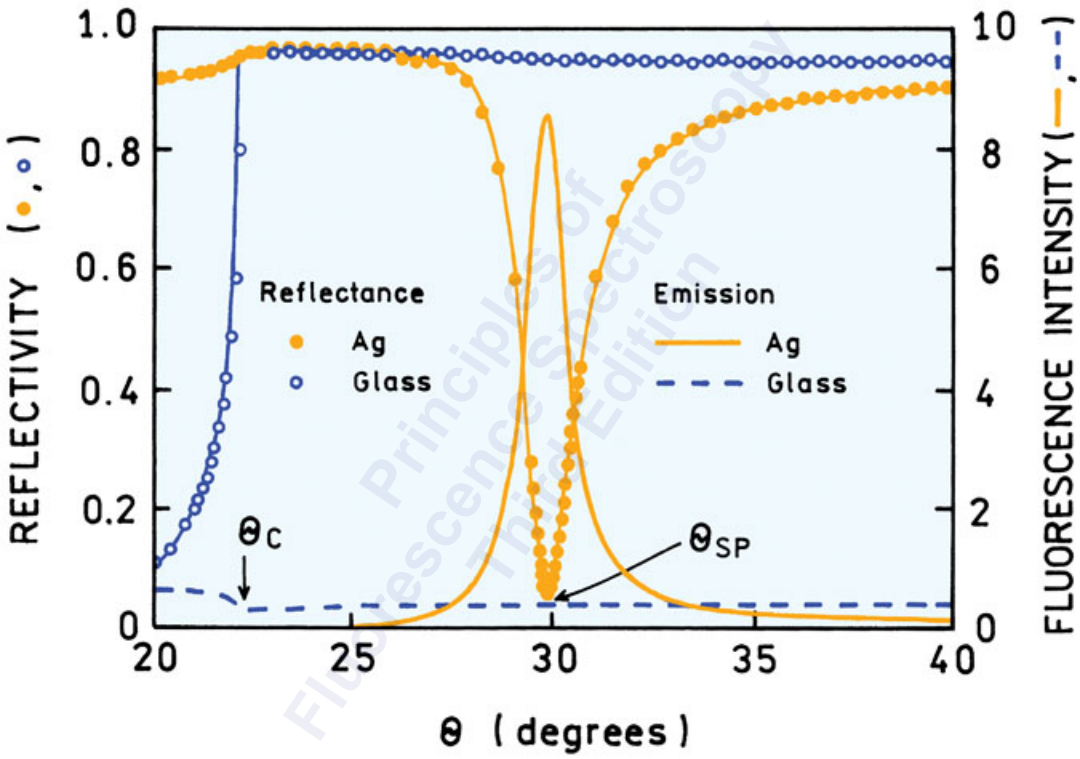


- Directional, polarized and spectrally resolved emission
- Requires minimal thickness of sample, ideal for monolayers and microscopy
- Background rejection from bulk solution
- New DNA and protein assays

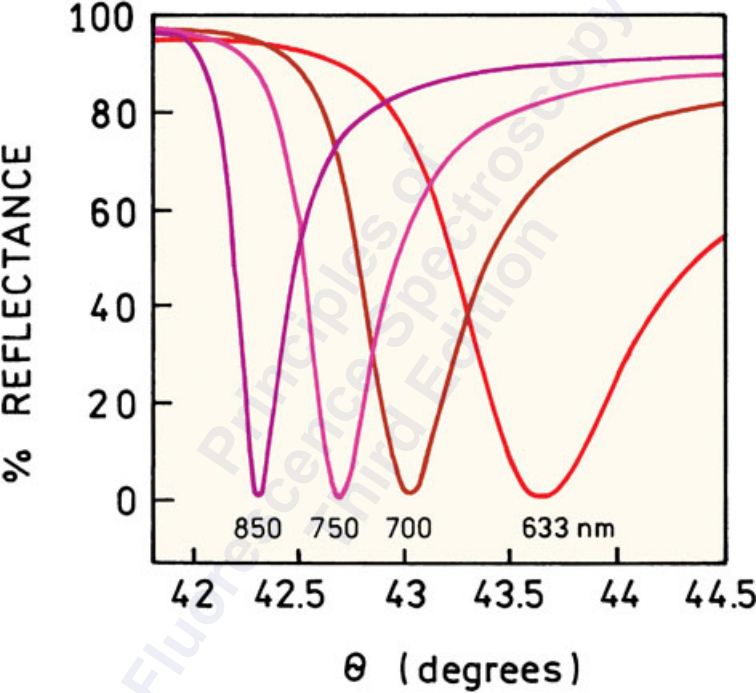


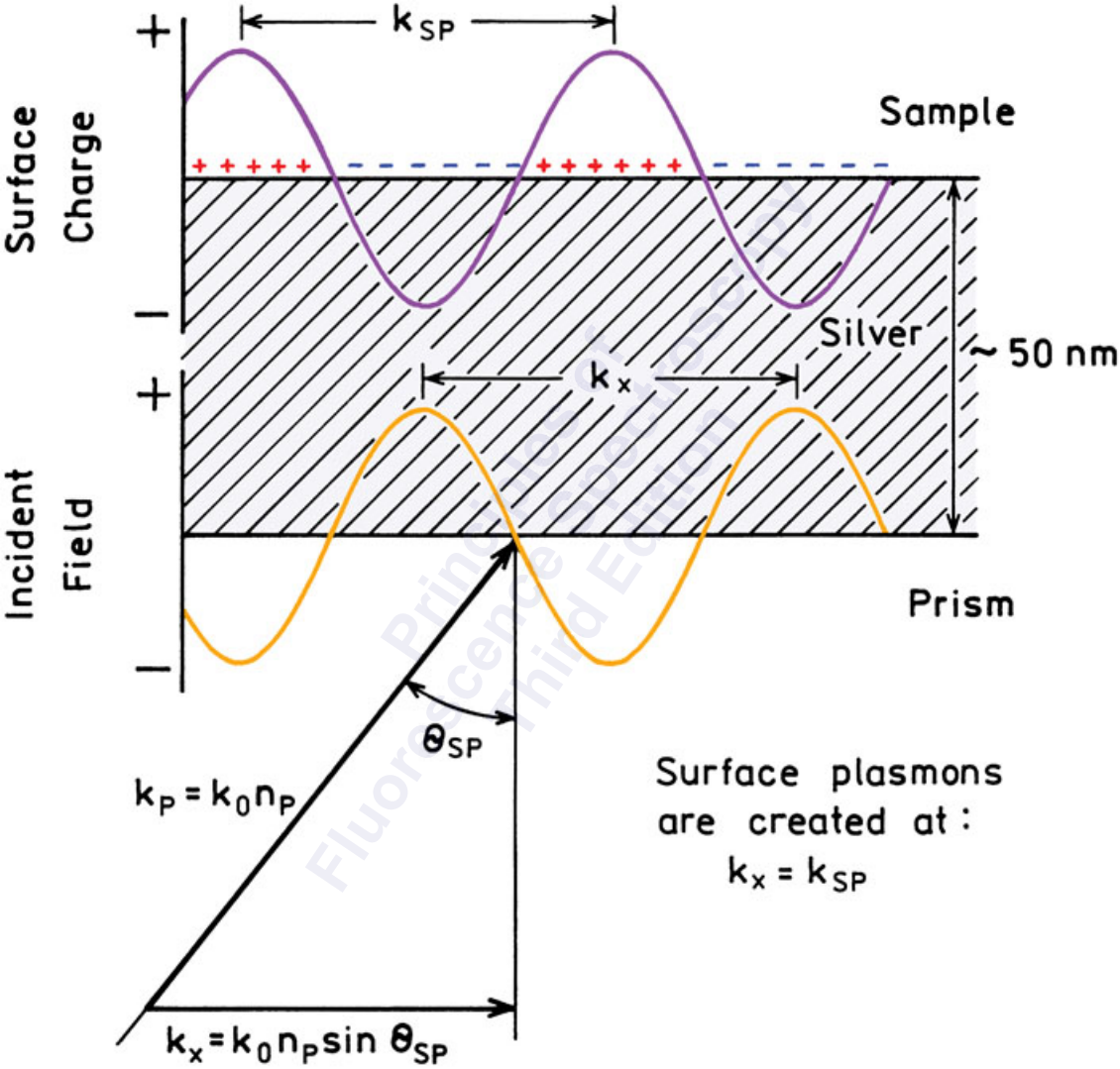


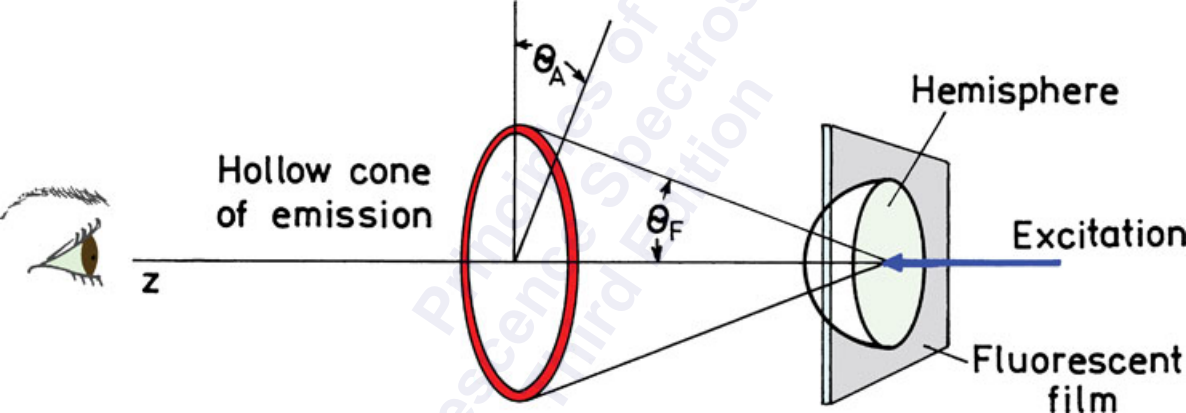


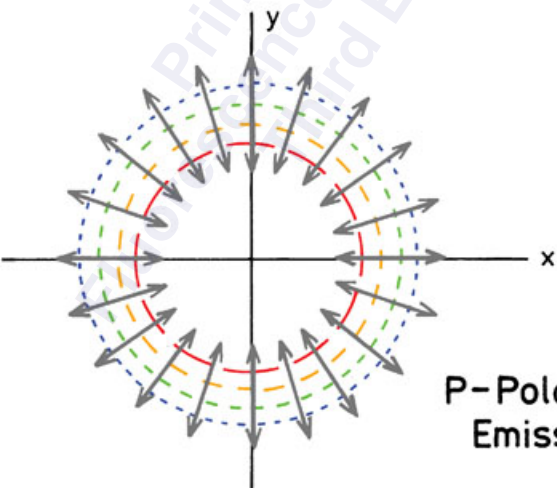
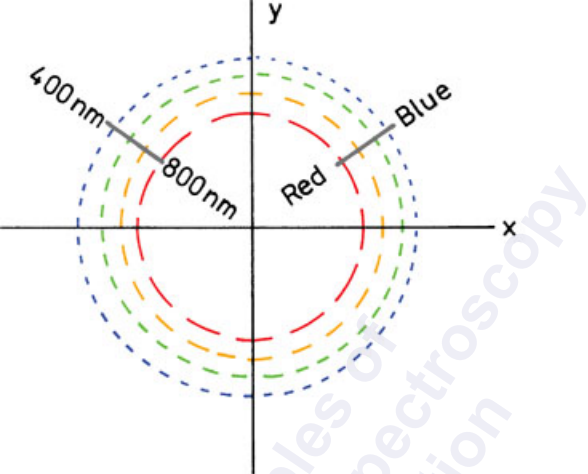




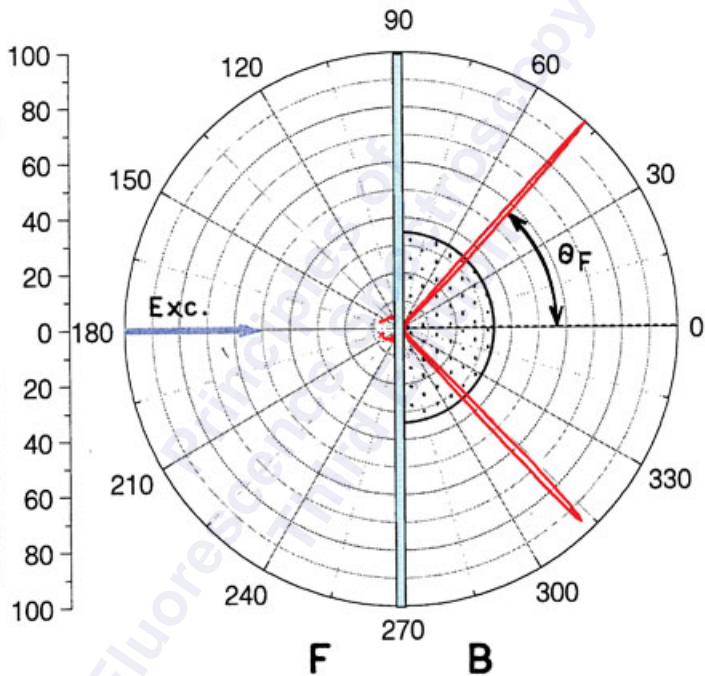


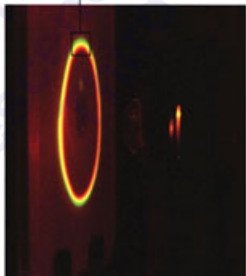
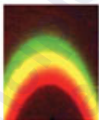
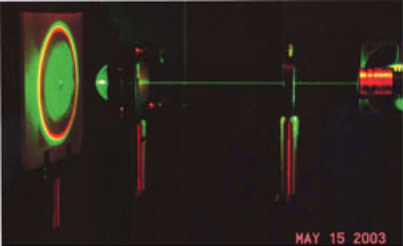






Normalized fluorescence intensity

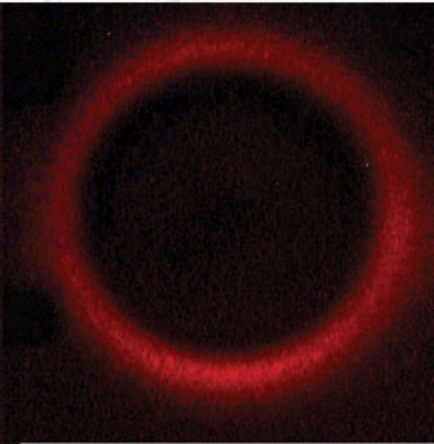




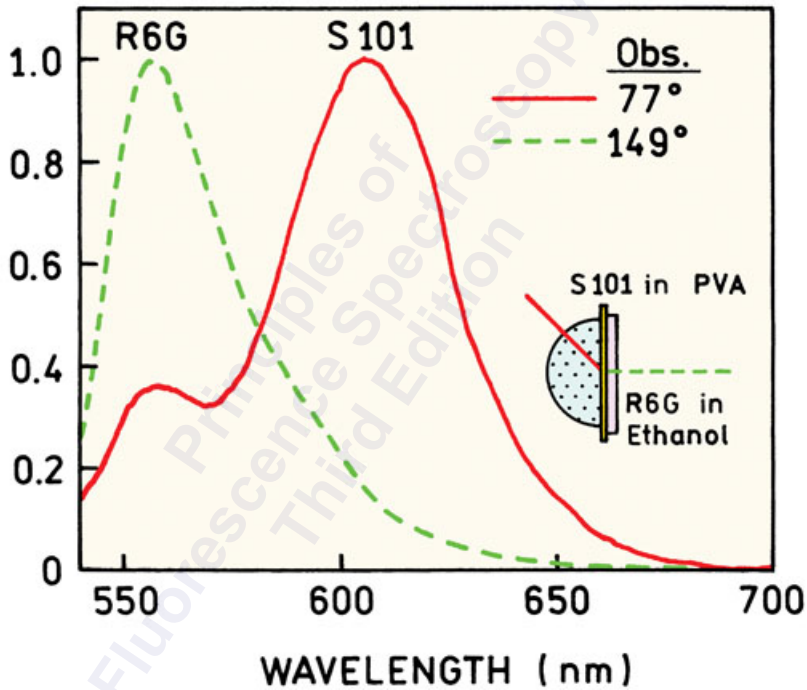
Silver



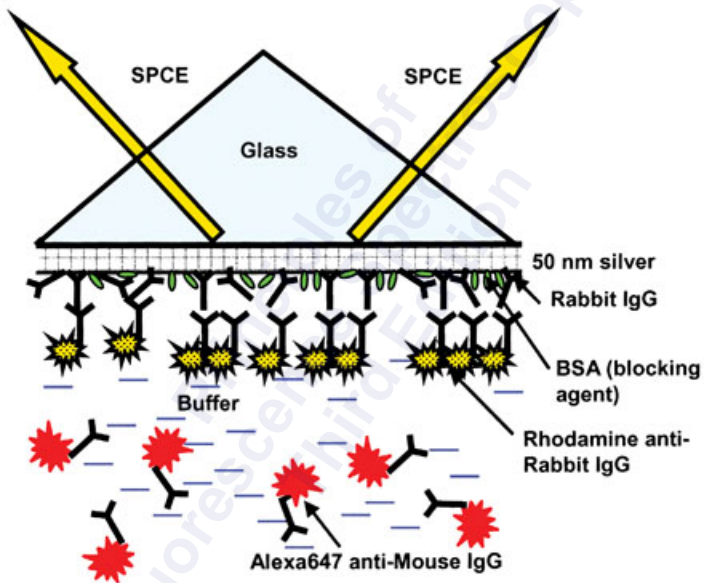
Gold



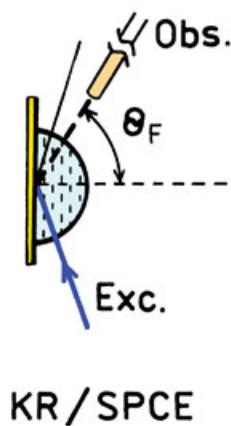
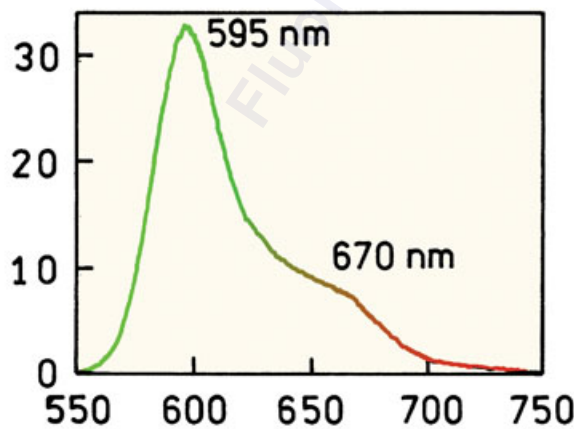
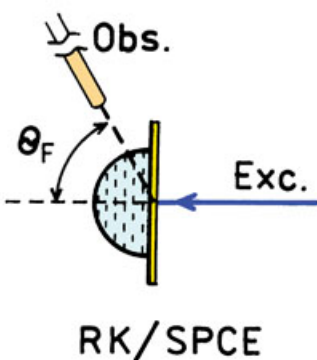
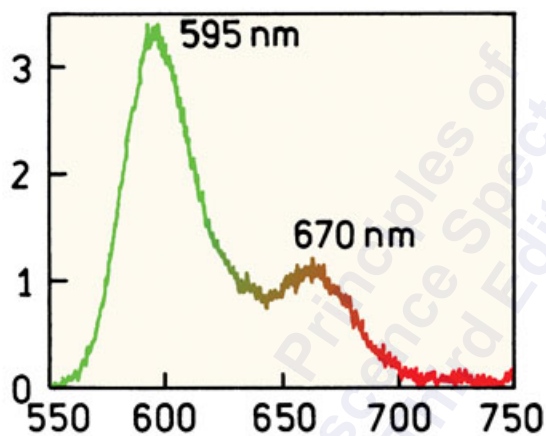
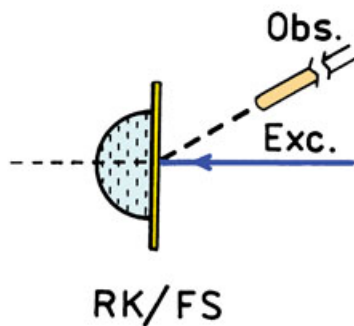
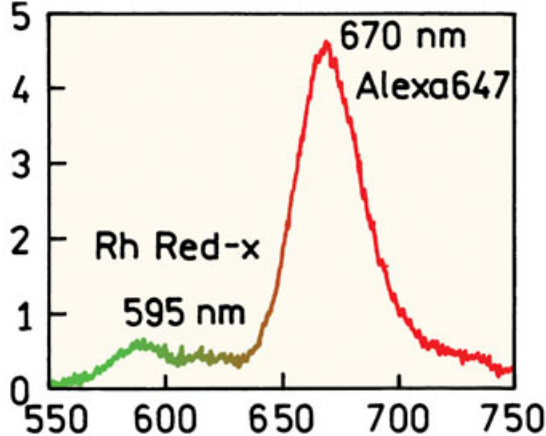
NORMALIZED  
FLUORESCENCE INTENSITY



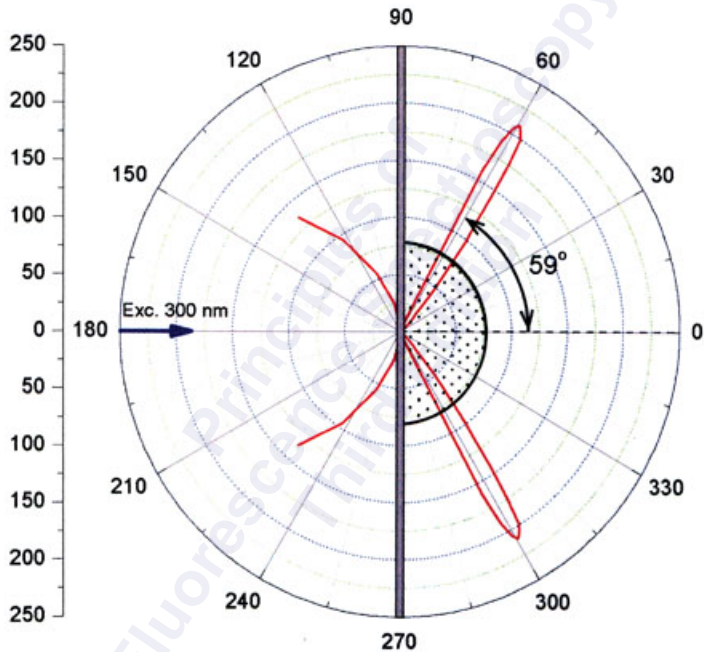


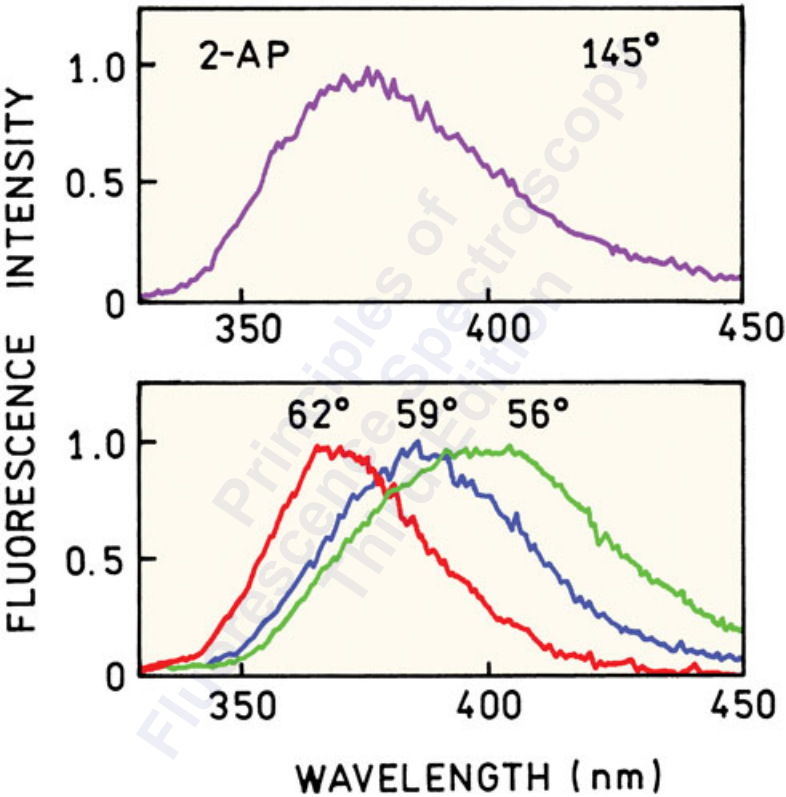


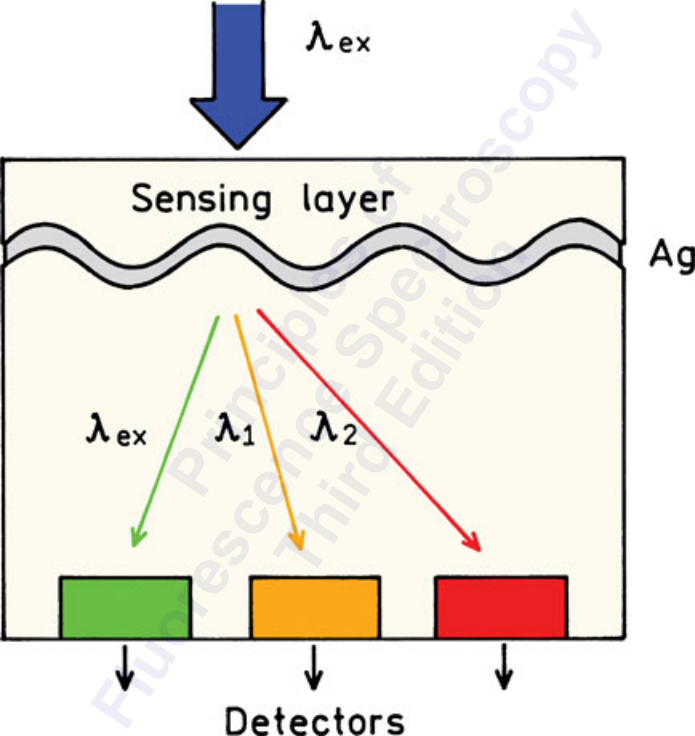
FLUORESCENCE INTENSITY, a.u.



WAVELENGTH (nm)



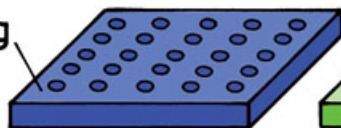




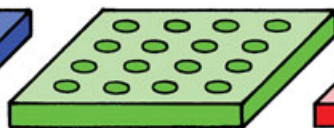
White Light



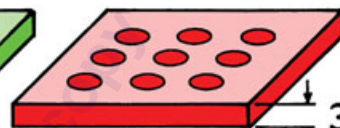
Ag



$s = 300 \text{ nm}$   
 $d = 155 \text{ nm}$



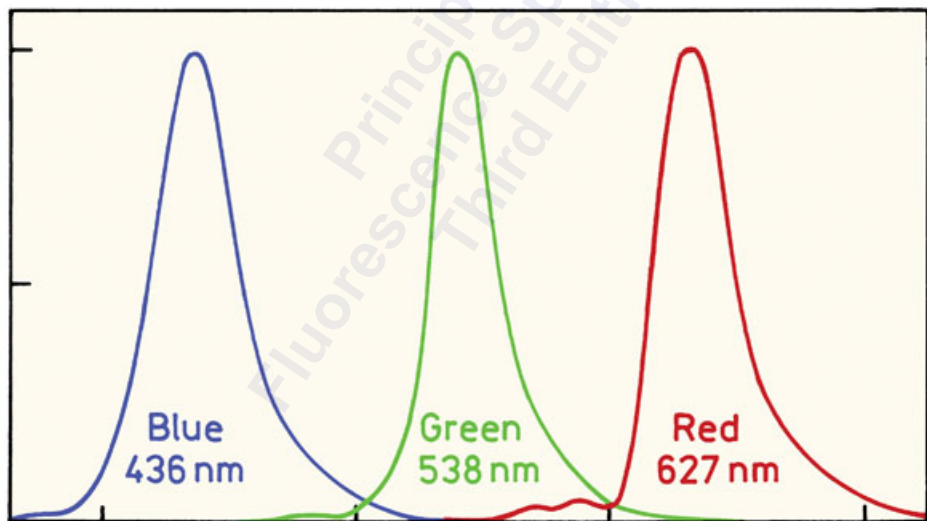
$s = 450 \text{ nm}$   
 $d = 180 \text{ nm}$



$s = 550 \text{ nm}$   
 $d = 225 \text{ nm}$

300 nm

TRANSMITTED INTENSITY



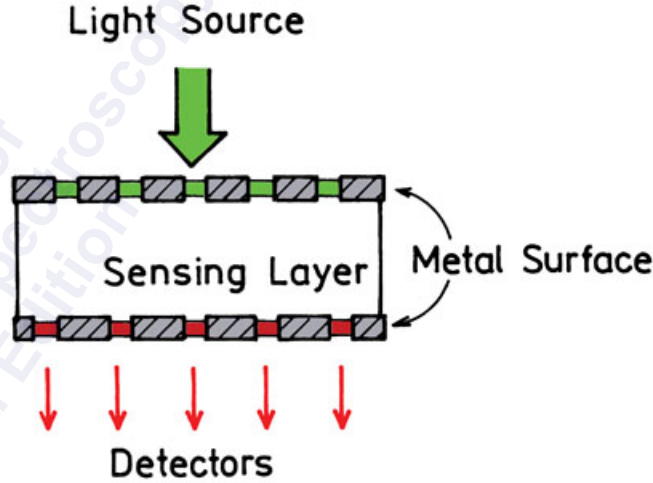
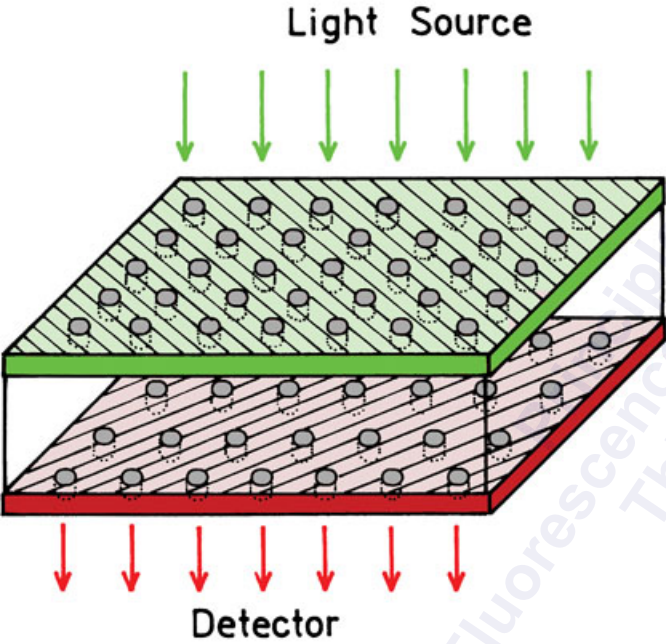
400

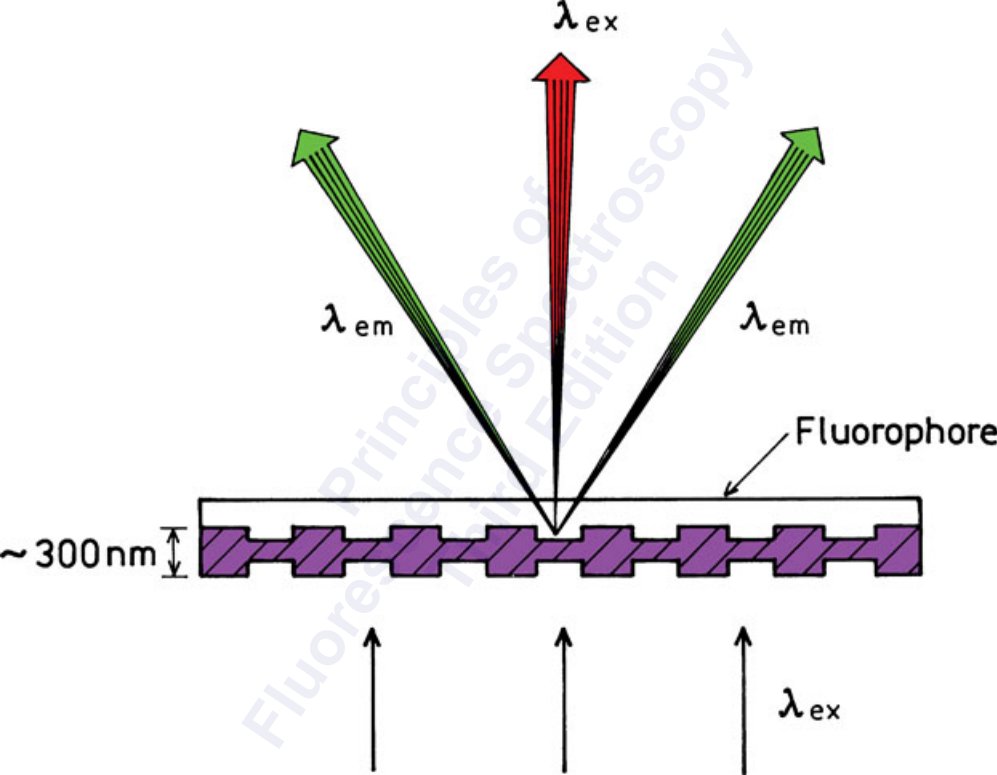
500

600

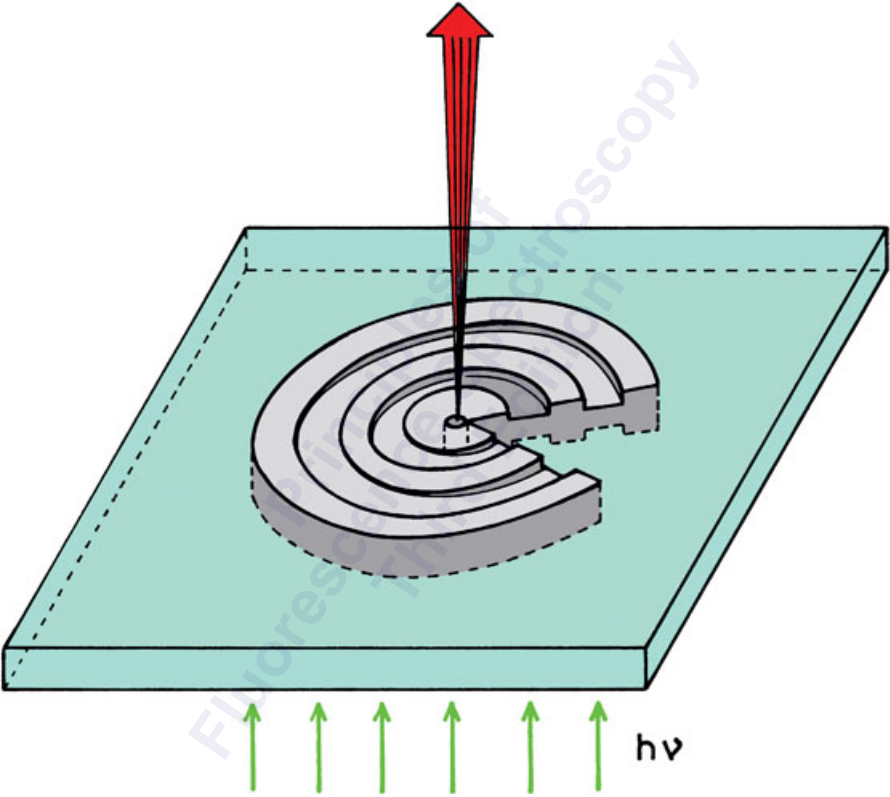
700

WAVELENGTH (nm)



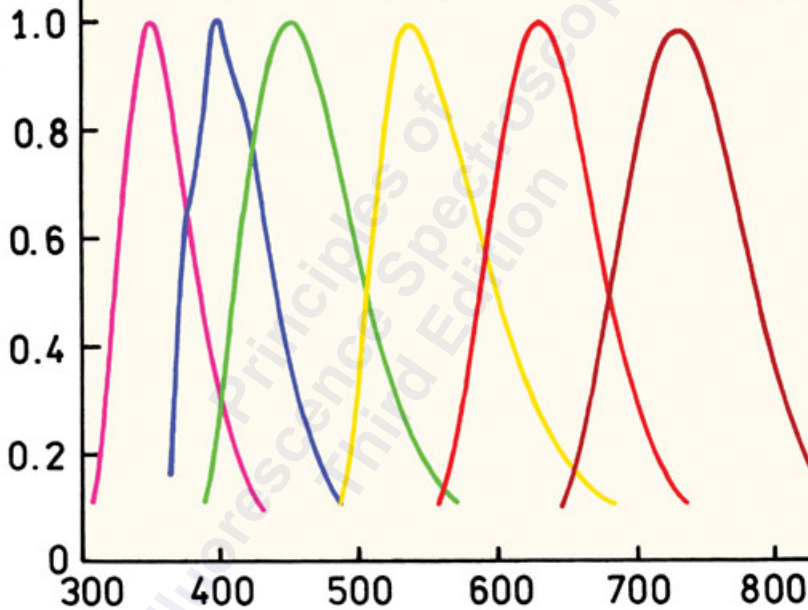




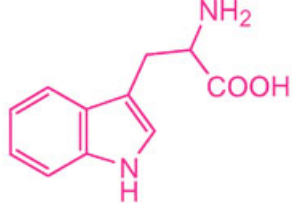


INTENSITY ( photons / nm )

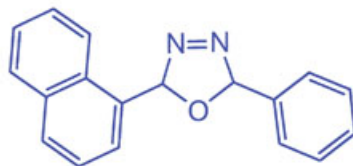
Tryptophan TPB DCM  
 $\alpha$ -NPO Coumarin 153 LDS 751



WAVELENGTH ( nm )



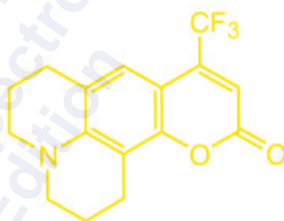
Trp



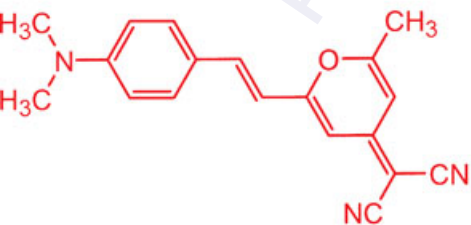
$\alpha$ -NPO



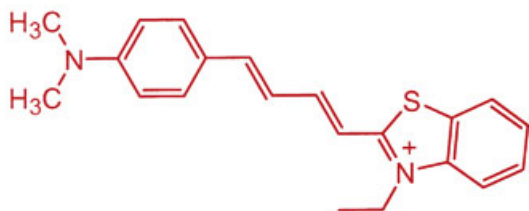
TBP



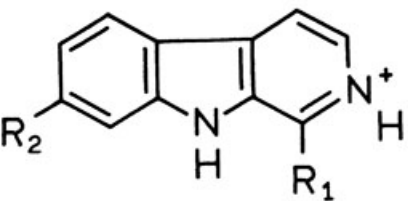
Coumarin 153



DCM



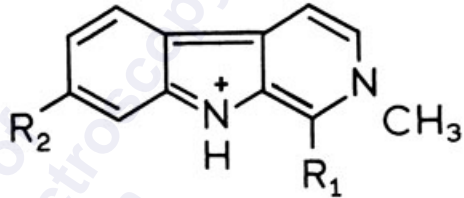
LDS 751



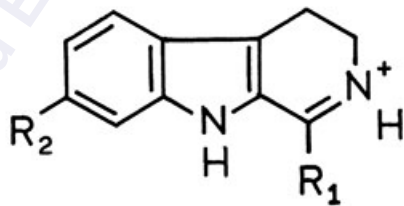
$R_1 = H$ ,  $R_2 = H$  (a)

$R_1 = CH_3$ ,  $R_2 = H$  (b)

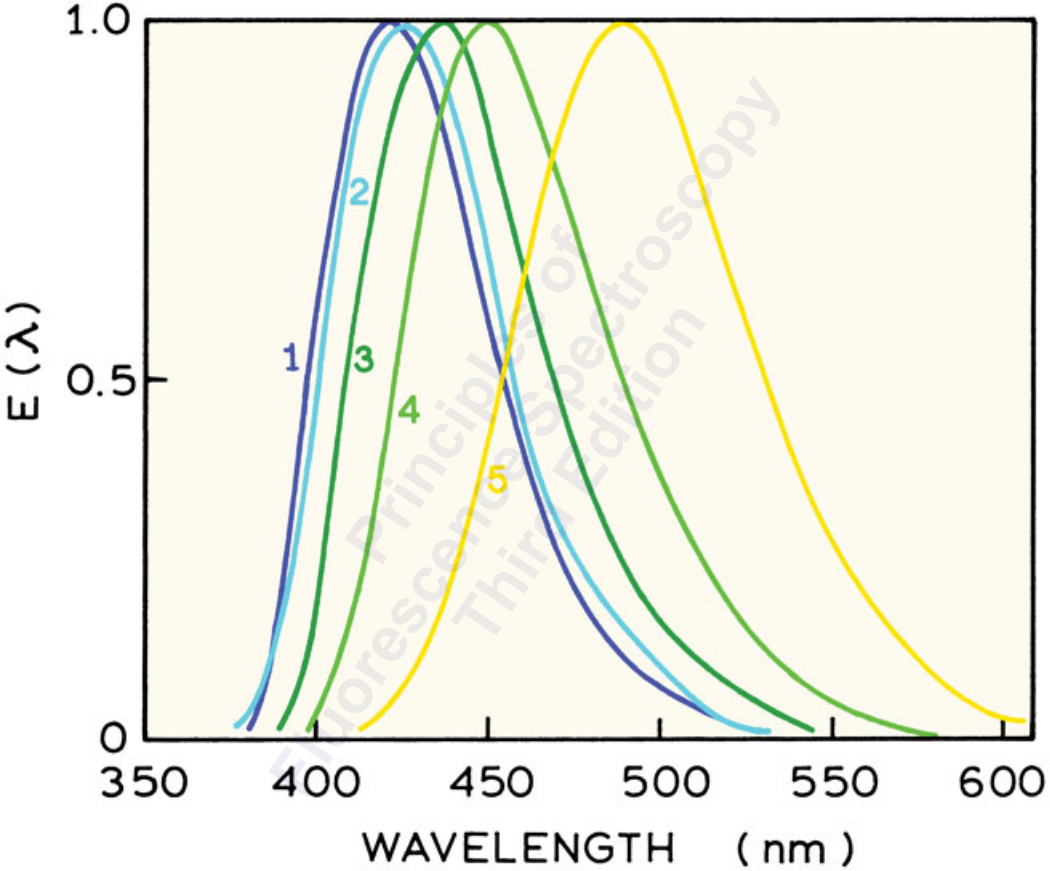
$R_1 = CH_3$ ,  $R_2 = OCH_3$  (c)

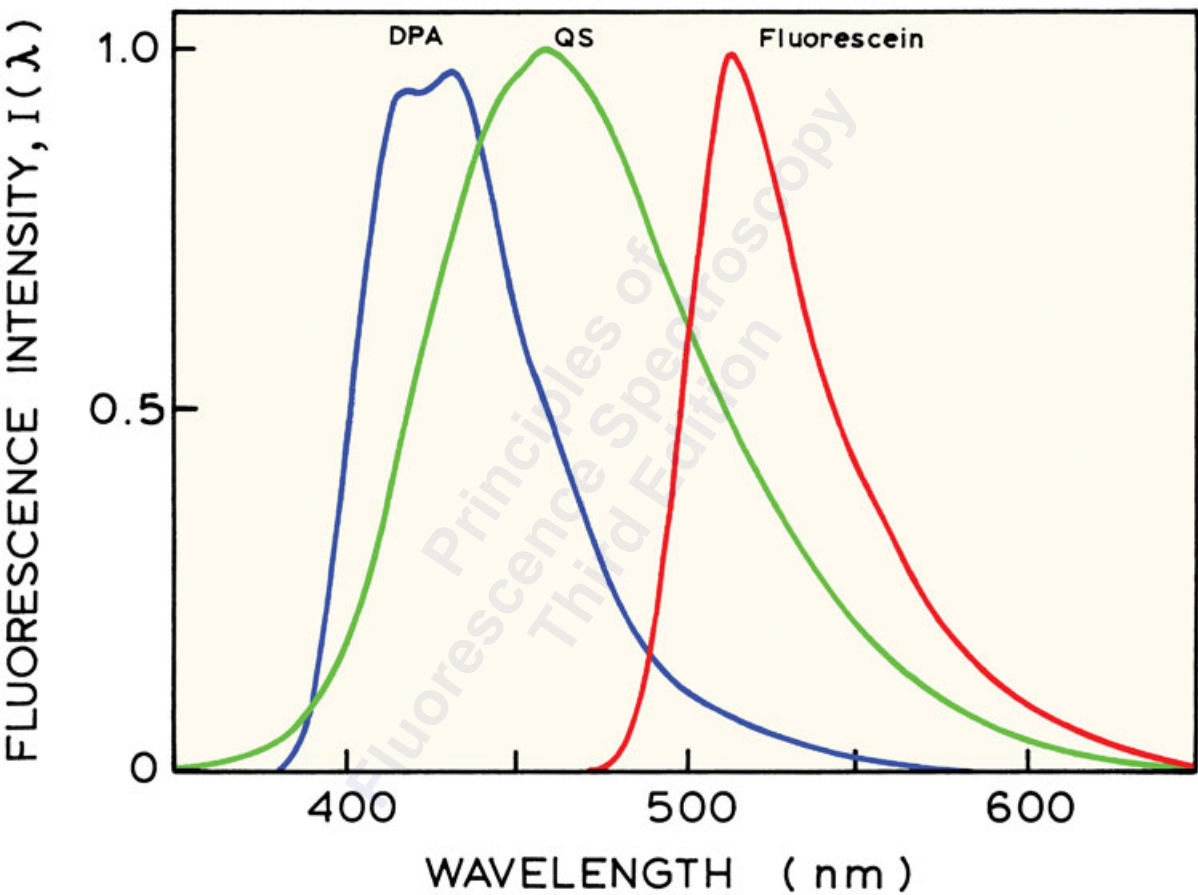


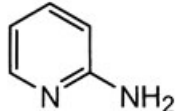
$R_1 = CH_3$ ,  $R_2 = OCH_3$  (d)



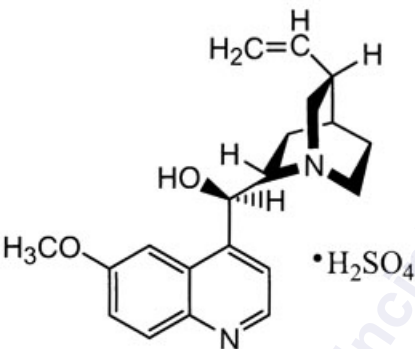
$R_1 = CH_3$ ,  $R_2 = OCH_3$  (e)



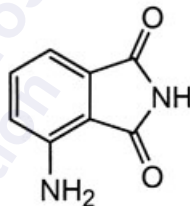




2-AP



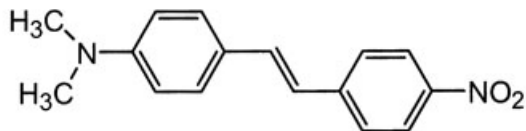
Quinine Sulfate



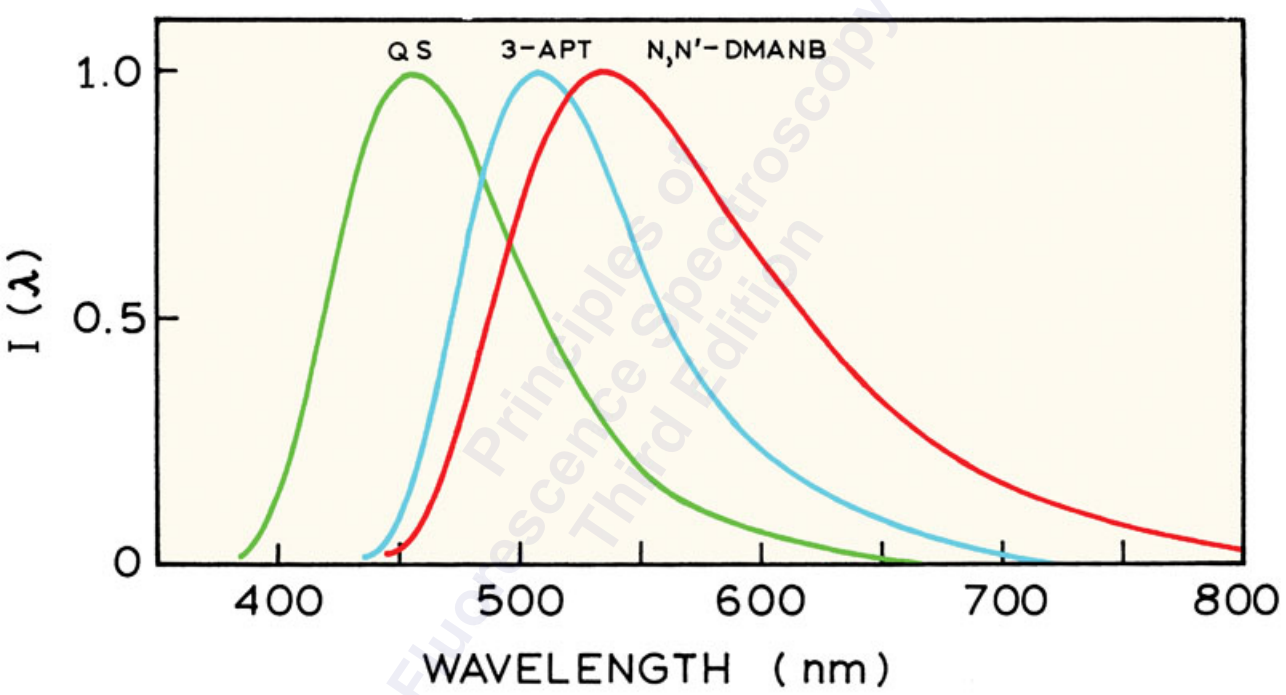
3-APT



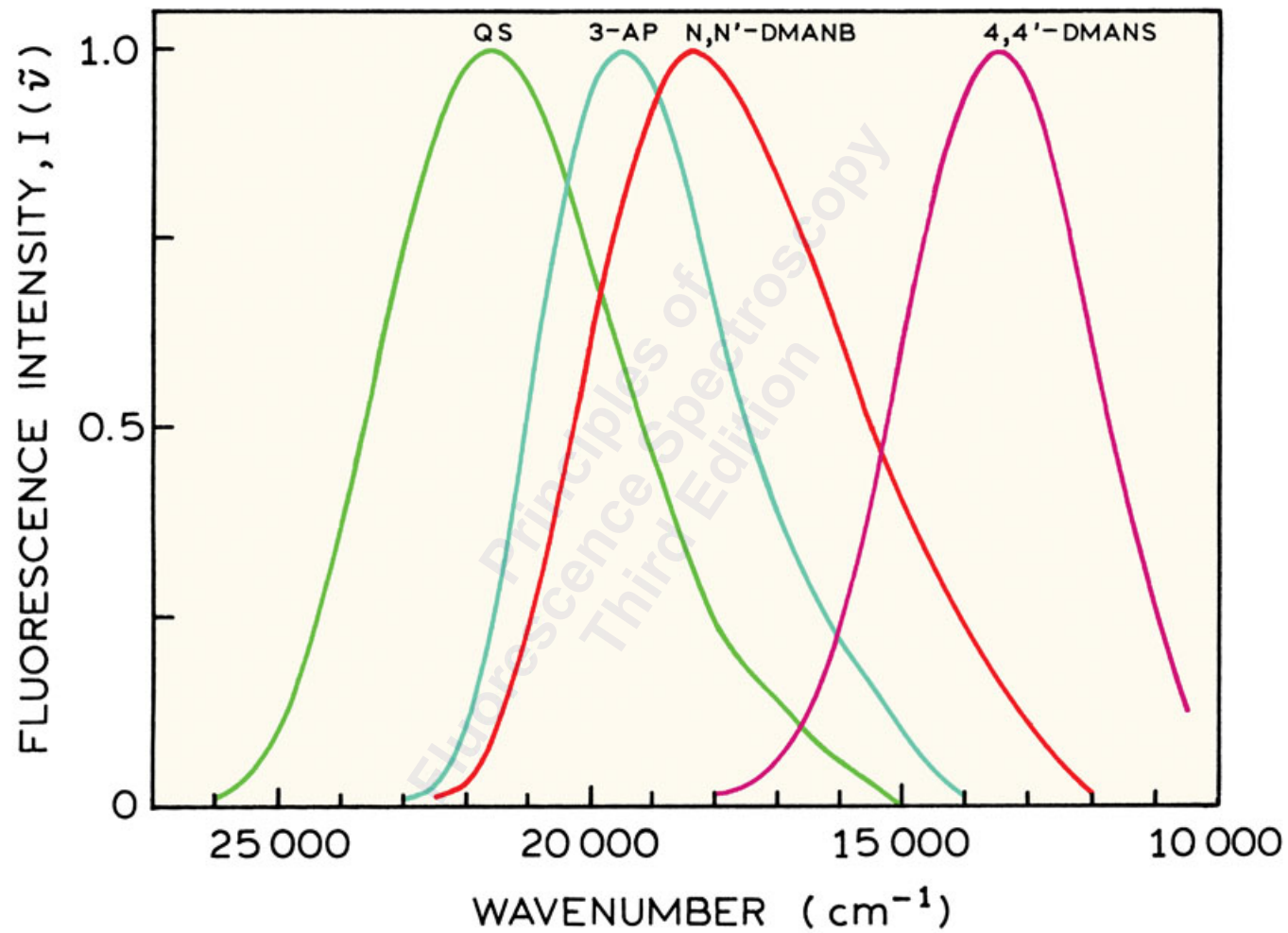
N,N-DMANB

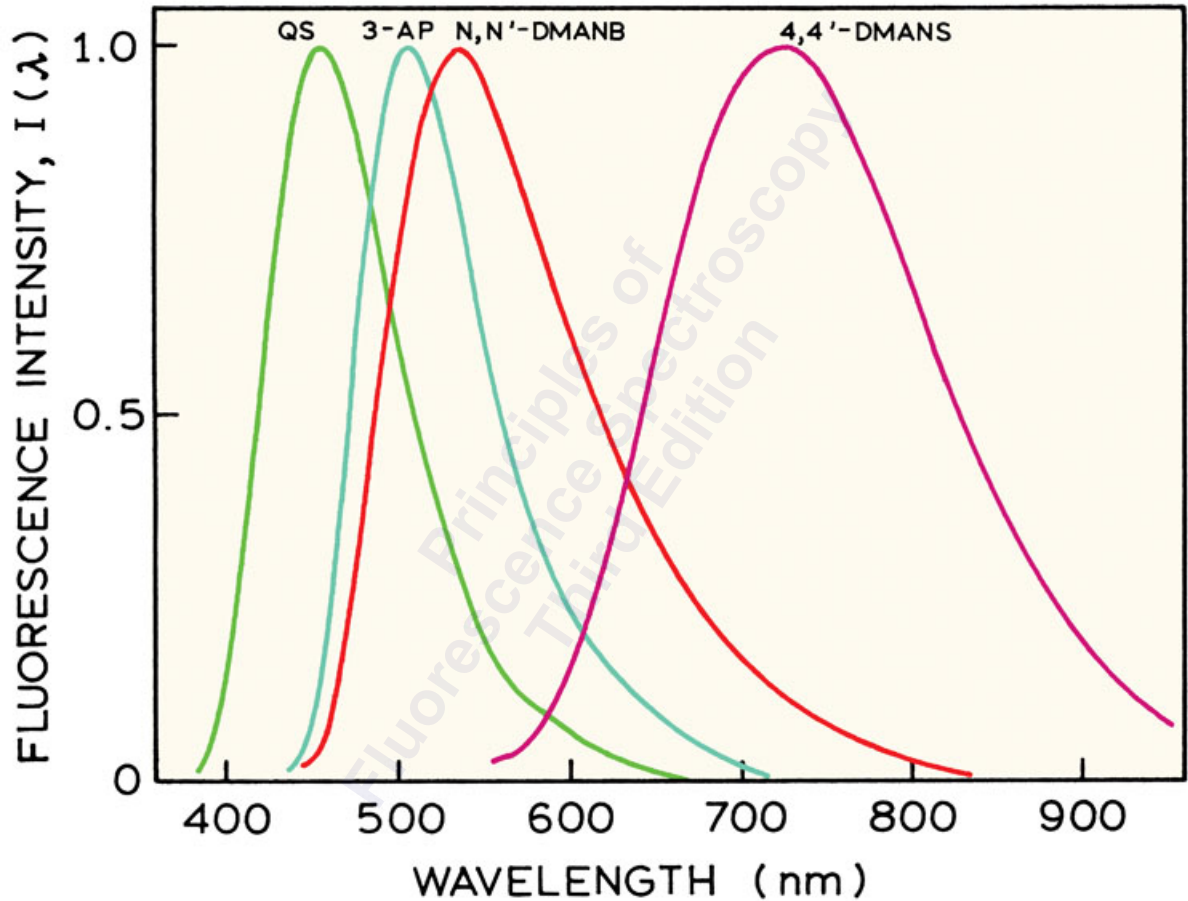


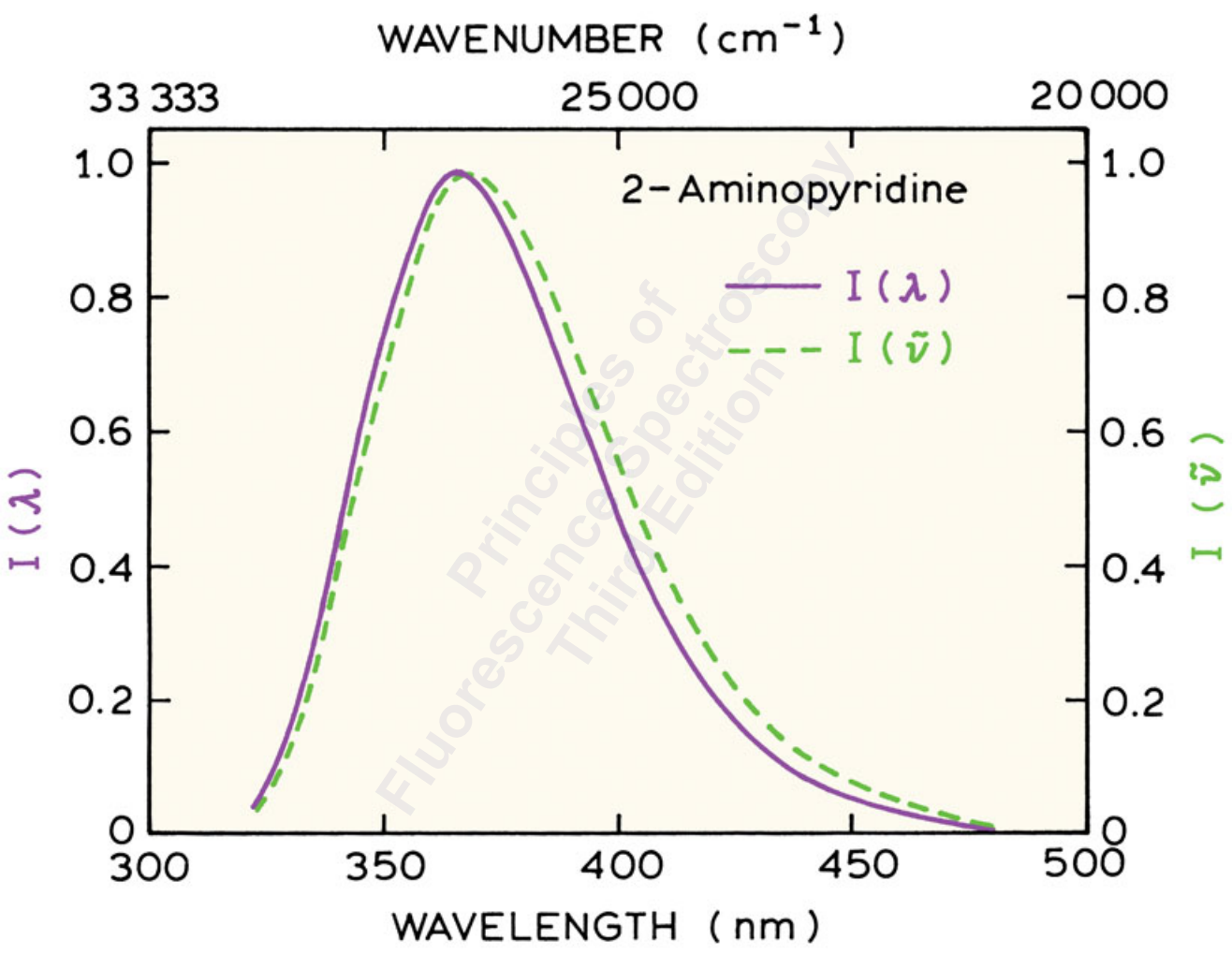
4,4'-DMANS

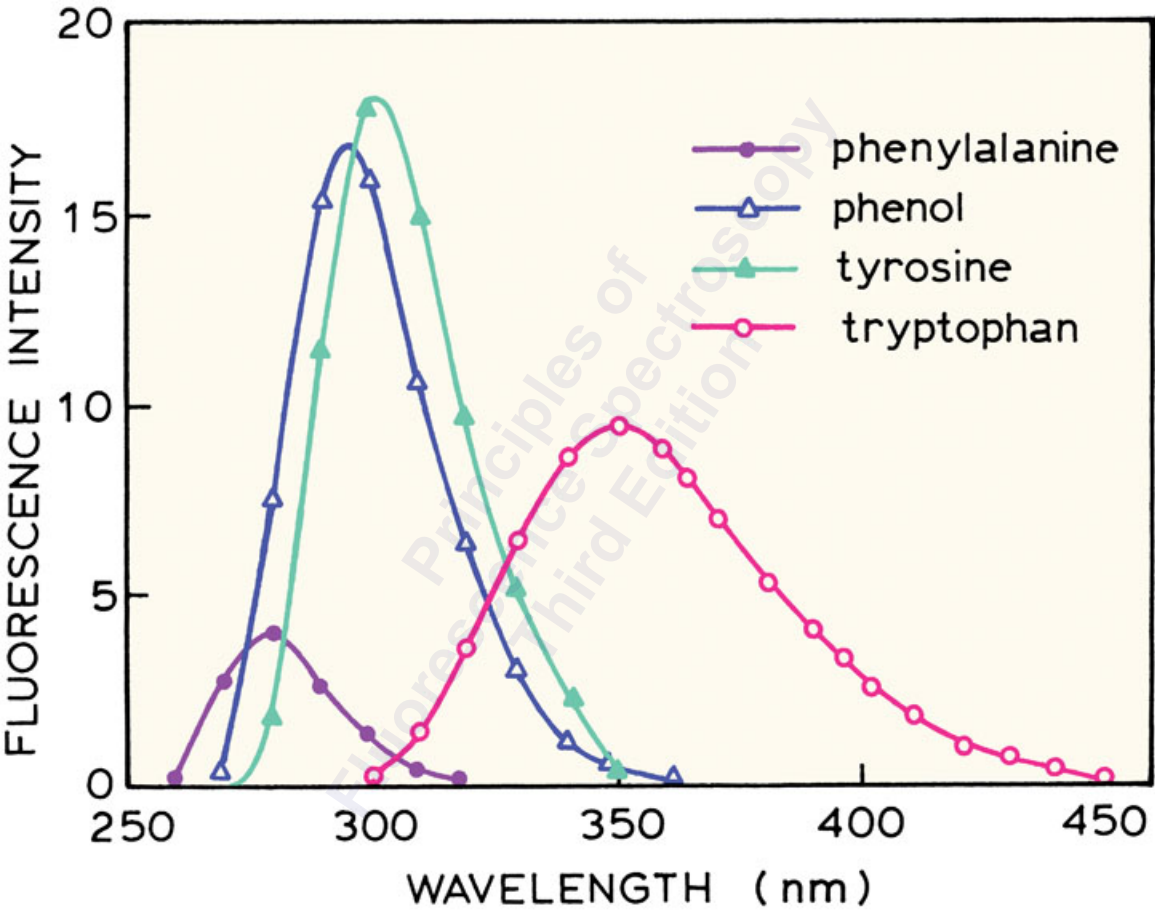


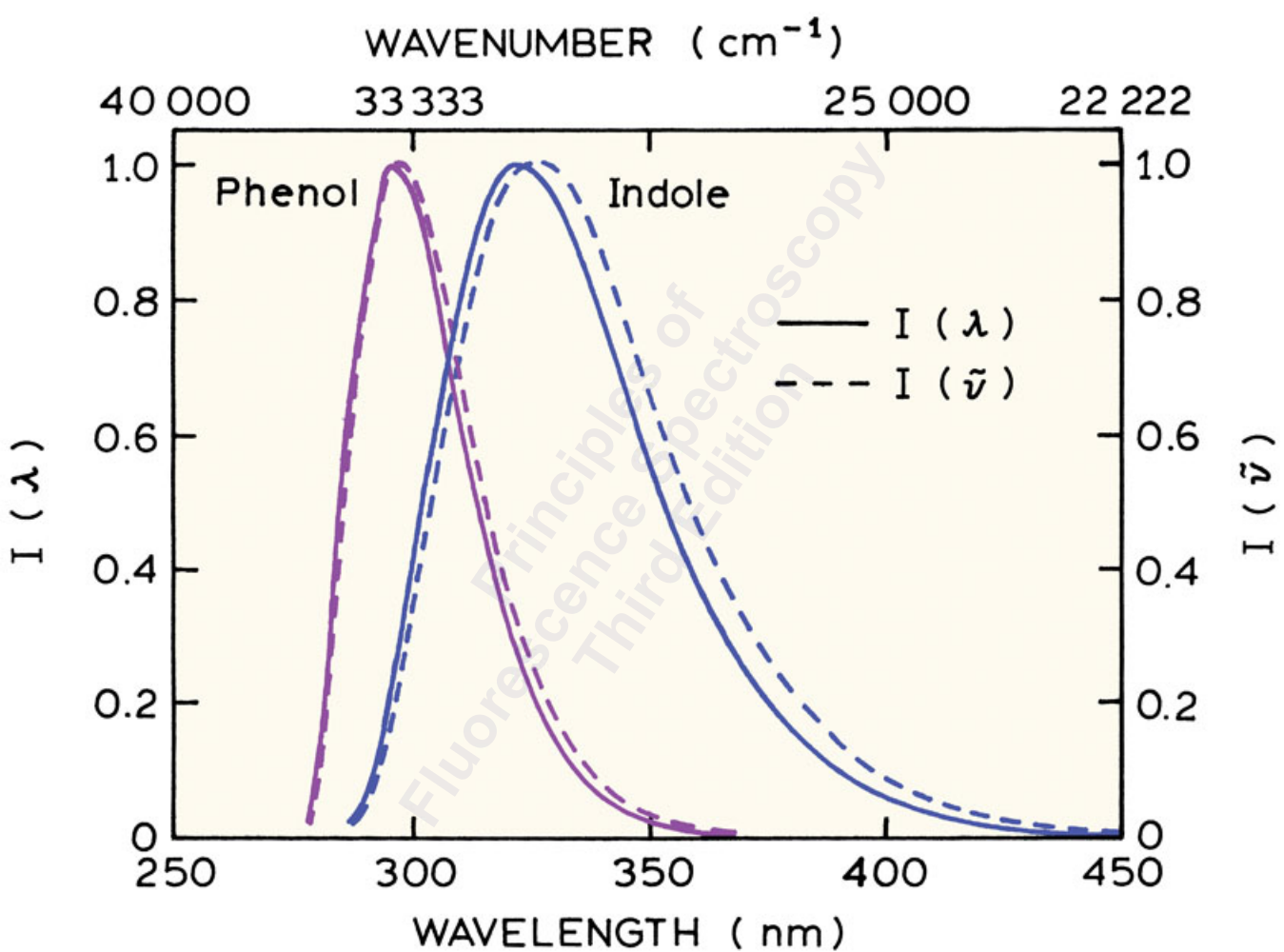




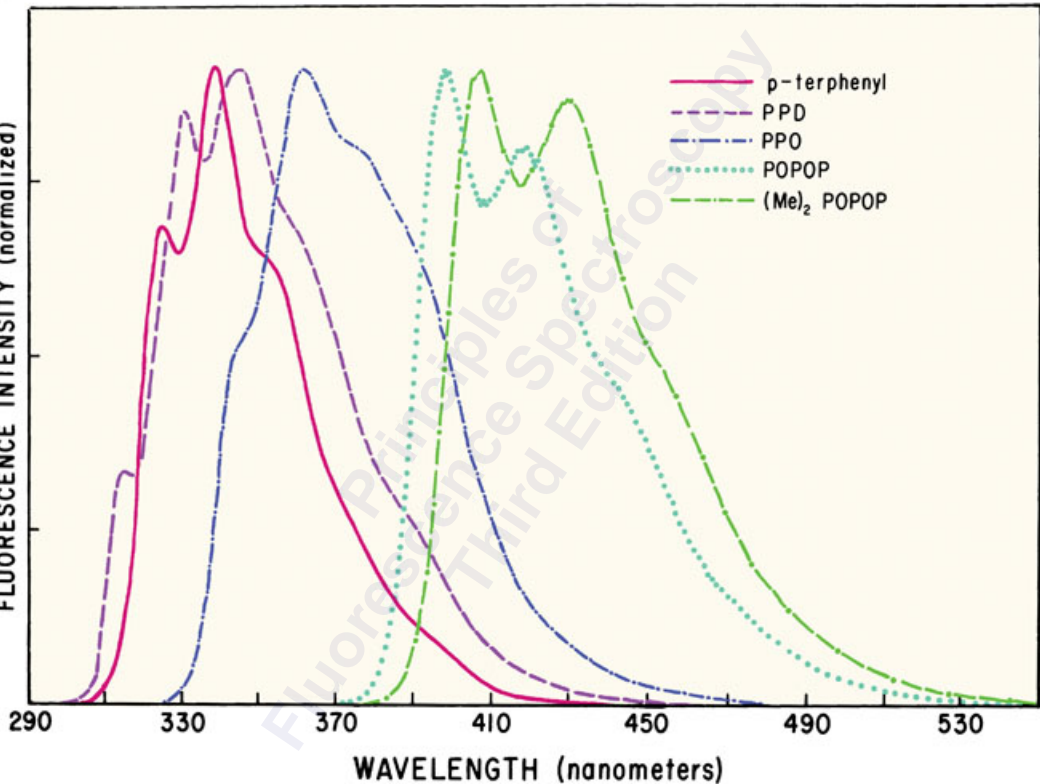


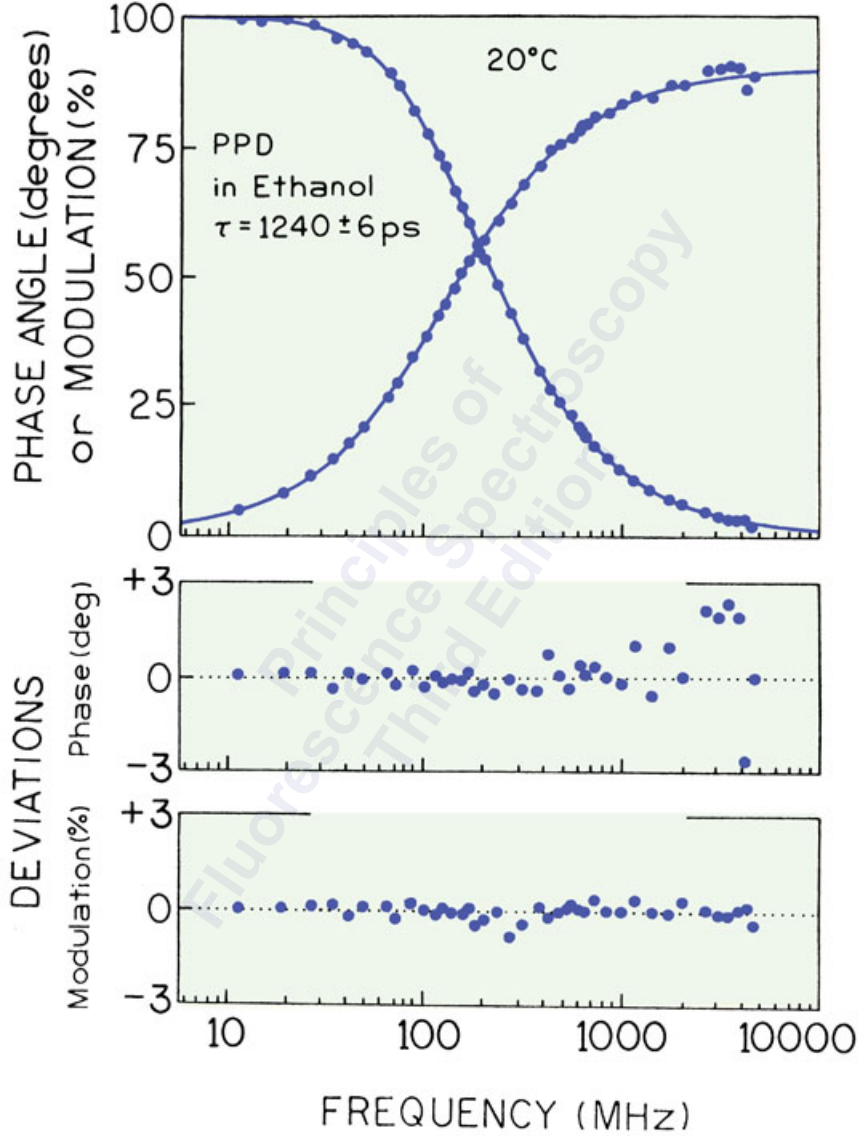


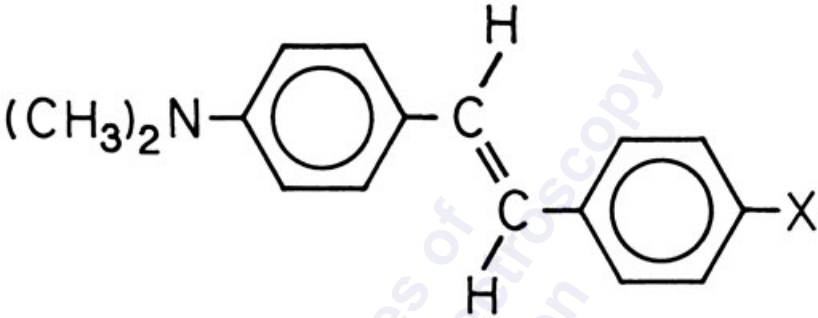




FLUORESCENCE INTENSITY (normalized)







	X
DMS	$\text{OCH}_3$
DFS	F
DBS	Br
DCS	CN



